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(54) Titre : ACIDE NUCLEIQUE, COMPOSITION PHARMACEUTIQUE, CONJUGUE, PROCEDE DE PREPARATION ET UTILISATION

(54) Title: NUCLEIC ACID, PHARMACEUTICAL COMPOSITION, CONJUGATE, PREPARATION METHOD, AND USE

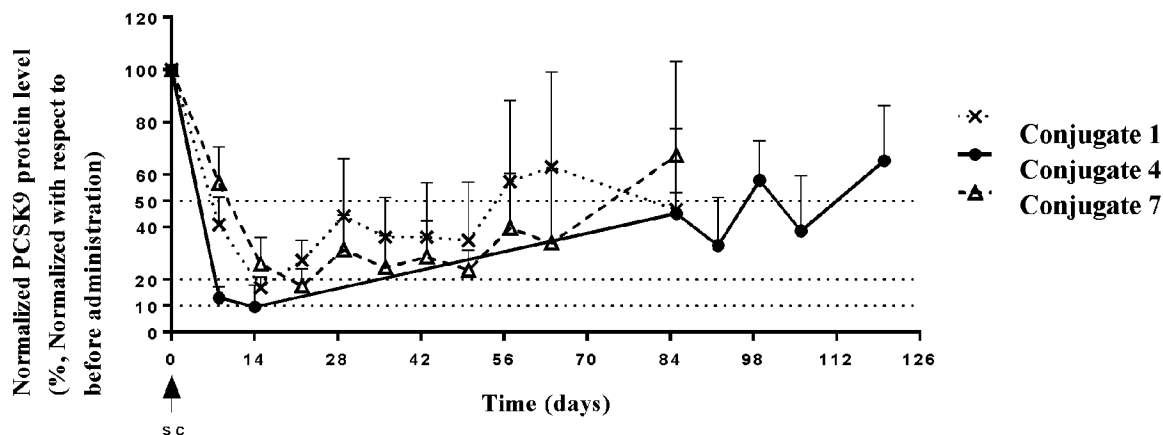


Figure 2

(57) **Abrégé/Abstract:**

An siRNA which inhibits proprotein convertase subtilisin/kexin type 9 (PCSK9) gene expression, a pharmaceutical composition containing the siRNA, and a conjugate. Each nucleotide in the siRNA is independently a modified or unmodified nucleotide. The siRNA contains a sense strand and an antisense strand. The sense strand contains nucleotide sequence I, nucleotide sequence I having the same length as the nucleotide sequence shown in SEQ ID NO: 1, with no more than three nucleotide differences. The antisense strand contains nucleotide sequence II, nucleotide sequence II having the same length as the nucleotide sequence shown in SEQ ID NO: 2, with no more than three nucleotide differences. The siRNA, the pharmaceutical composition thereof and the conjugate can effectively treat and/or prevent hypercholesterolemia.

ABSTRACT

5 An siRNA which inhibits proprotein convertase subtilisin/kexin type 9 (PCSK9) gene expression, a pharmaceutical composition containing the siRNA, and a conjugate. Each nucleotide in the siRNA is independently a modified or unmodified nucleotide. The siRNA contains a sense strand and an antisense strand. The sense strand contains nucleotide sequence I; nucleotide sequence I having the same length as the nucleotide sequence shown in SEQ ID NO: 1, with no more than three nucleotides differences. The antisense strand contains nucleotide sequence II, nucleotide sequence
10 II having the same length as the nucleotide sequence shown in SEQ ID NO: 2, with no more than three nucleotides differences. The siRNA, pharmaceutical composition thereof and the conjugate can effectively treat and/or prevent hypercholesterolemia.

NUCLEIC ACID, PHARMACEUTICAL COMPOSITION, CONJUGATE, PREPARATION METHOD, AND USE

TECHNICAL FIELD

5 The present disclosure relates to a nucleic acid capable of inhibiting the expression of proprotein convertase subtilisin/kexin type 9 (PCSK9) gene, a pharmaceutical composition and an siRNA conjugate. The present disclosure also relates to a preparation method and use of such nucleic acids, pharmaceutical compositions and siRNA conjugates.

10 BACKGROUND ART

Dyslipidemia, especially persistently high levels of low-density lipoprotein cholesterol (LDL-c), is an important risk factor that induces and promotes the occurrence and development of atherosclerosis, and is closely related to ischemic cardiovascular and cerebrovascular diseases, posing a serious threat to human health. The currently available medicines for treating dyslipidemia mainly include statins, cholesterol absorption inhibitors, resins, probucol, fibrates, niacins, and derivatives thereof. The application of lipid-lowering medicines for lowering plasma cholesterol levels can reduce the risk of cardiovascular and cerebrovascular diseases. Nevertheless, there is still a considerable proportion of patients who fail to achieve desired lipid levels.

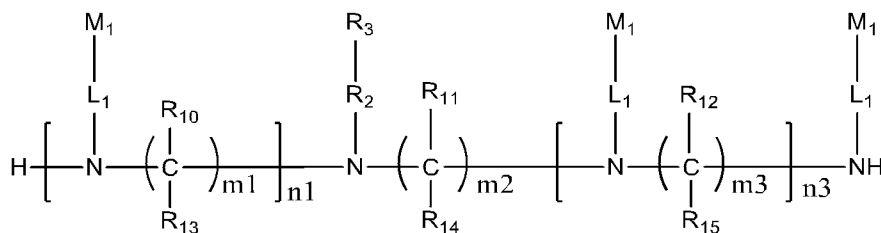
Studies show that the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene plays an important role in lipid metabolism, especially cholesterol metabolism. By inhibiting the expression of PCSK9 gene, plasma cholesterol levels can be effectively reduced. Based on the mechanism of RNA interference (RNAi), small interfering RNA (siRNA) could inhibit or block the expression of a target gene of interest in a sequence-specific manner, so as to achieve the purpose of treating diseases. Inhibiting the expression of PCSK9 gene at mRNA level will undoubtedly be the most ideal treatment means.

The key to developing siRNA medicines that inhibit the expression of PCSK9 gene and treat hypercholesterolemia is to find suitable siRNA and its modification and effective delivery system.

30 SUMMARY OF THE INVENTION

The inventors of the present disclosure have surprisingly found that the siRNA conjugate provided by the present disclosure below is capable of specifically inhibiting the expression of PCSK9 gene, and specifically targeting the liver and inhibiting the expression of PCSK9 gene in the liver, thereby achieving the treatment or prevention of hypercholesterolemia. In addition, the inventors have further invented siRNAs with higher activity and pharmaceutical compositions.

In some embodiments, the present disclosure provides an siRNA conjugate having a structure as shown by Formula (308):



Formula (308),

wherein

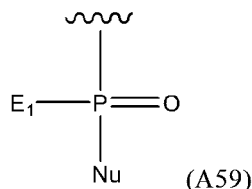
n1 is an integer of 1-3, and n3 is an integer of 0-4;

m1, m2, or m3 independently of one another is an integer of 2-10;

R₁₀, R₁₁, R₁₂, R₁₃, R₁₄, or R₁₅ independently of one another is H, or selected from the group consisting of C₁-

5 C₁₀ alkyl, C₁-C₁₀ haloalkyl, and C₁-C₁₀ alkoxy;

R₃ is a group having a structure as shown by Formula (A59):



wherein,

E₁ is OH, SH or BH₂;

10 Nu is an siRNA;

the siRNA comprises a sense strand and an antisense strand; each nucleotide in the siRNA is independently a modified or unmodified nucleotide; wherein the sense strand comprises a nucleotide sequence I, and the antisense strand comprises a nucleotide sequence II; the nucleotide sequence I and the nucleotide sequence II are at least partly reverse complementary to form a double-stranded region; the nucleotide sequence I and the

15 nucleotide sequence II are the sequences selected from one of the following groups i)-vi):

i) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 1 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 2 have an equal length and no more than 3 nucleotide differences:

20 5'- AAGCAAGCAGACAUUUAU_{Z1} -3' (SEQ ID NO: 1);
5'- Z₂AUAAAUGUCUGCUUGCUU -3' (SEQ ID NO: 2),

wherein, Z₁ is C and Z₂ is G, and

the nucleotide sequence I comprises a nucleotide Z₃ at the position corresponding to Z₁; the nucleotide sequence

25 II comprises a nucleotide Z₄ at the position corresponding to Z₂, wherein Z₄ is the first nucleotide at 5' terminal of the antisense strand;

ii) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 61 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 62 have an equal length and no more than 3 nucleotide differences:

30 5'- UUUGUAGCAUUUUUAUUAZ₅ -3' (SEQ ID NO: 61);
5'- Z₆UAAUAAAAAUGCUACAAA -3' (SEQ ID NO: 62),

wherein, Z₅ is A and Z₆ is U, and

35 the nucleotide sequence I comprises a nucleotide Z₇ at the position corresponding to Z₅; the nucleotide sequence II comprises a nucleotide Z₈ at the position corresponding to Z₆, wherein Z₈ is the first nucleotide at 5' terminal of the antisense strand;

iii) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 121 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 122 have an equal length and no more than 3 nucleotide differences:

5'- GCCUGGAGUUAUUCGGAZ₉ -3' (SEQ ID NO: 121);

5'-Z₁₀UCCGAAUAAACUCCAGGC-3' (SEQ ID NO: 122),

wherein, Z₉ is A and Z₁₀ is U, and

the nucleotide sequence I comprises a nucleotide Z₁₁ at the position corresponding to Z₉; the nucleotide
 5 sequence II comprises a nucleotide Z₁₂ at the position corresponding to Z₁₀, wherein Z₁₂ is the first nucleotide
 at 5' terminal of the antisense strand;

iv) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 181 have an equal length
 and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown
 10 by SEQ ID NO: 182 have an equal length and no more than 3 nucleotide differences:

5'-CUGUUUUGCUUUUGUAAACZ₁₃-3' (SEQ ID NO: 181);

5'-Z₁₄GUUACAAAAGCAAAACAG-3' (SEQ ID NO: 182),

wherein, Z₁₃ is U and Z₁₄ is A, and

15 the nucleotide sequence I comprises a nucleotide Z₁₅ at the position corresponding to Z₁₃; the nucleotide
 sequence II comprises a nucleotide Z₁₆ at the position corresponding to Z₁₄, wherein Z₁₆ is the first nucleotide
 at 5' terminal of the antisense strand;

v) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 241 have an equal length
 20 and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown
 by SEQ ID NO: 242 have an equal length and no more than 3 nucleotide differences:

5'-GGUUUUGUAGCAUUUUUAZ₁₇-3' (SEQ ID NO: 241);

5'-Z₁₈UAAAAAUGCUACAAAACC-3' (SEQ ID NO: 242),

25 wherein, Z₁₇ is U and Z₁₈ is A, and

the nucleotide sequence I comprises a nucleotide Z₁₉ at the position corresponding to Z₁₇; the nucleotide
 sequence II comprises a nucleotide Z₂₀ at the position corresponding to Z₁₈, wherein Z₂₀ is the first nucleotide
 at 5' terminal of the antisense strand; and

30 vi) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 301 have an equal length
 and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown
 by SEQ ID NO: 302 have an equal length and no more than 3 nucleotide differences:

5'-GUGACUUUUUAAAAUAAAZ₂₁-3' (SEQ ID NO: 301);

5'-Z₂₂UUUAUUUUAAAAAGUCAC-3' (SEQ ID NO: 302),

35

wherein, Z₂₁ is A and Z₂₂ is U, and

the nucleotide sequence I comprises a nucleotide Z₂₃ at the position corresponding to Z₂₁; the nucleotide
 sequence II comprises a nucleotide Z₂₄ at the position corresponding to Z₂₂, wherein Z₂₄ is the first nucleotide
 at 5' terminal of the antisense strand;

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R₂ is a linear alkylene of 1 to 20 carbon atoms in length, wherein one or more carbon atoms are optionally
 replaced with any one or more groups selected from the group consisting of: C(O), NH, O, S, CH=N, S(O)₂,
 C₂-C₁₀ alkenylene, C₂-C₁₀ alkynylene, C₆-C₁₀ arylene, C₃-C₁₈ heterocyclylene, and C₅-C₁₀ heteroarylene; and
 wherein R₂ optionally has any one or more substituents selected from the group consisting of: C₁-C₁₀ alkyl, C₆-
 45 C₁₀ aryl, C₅-C₁₀ heteroaryl, C₁-C₁₀ haloalkyl, -OC₁-C₁₀ alkyl, -OC₁-C₁₀ alkylphenyl, -C₁-C₁₀ alkyl-OH, -OC₁-
 C₁₀ haloalkyl, -SC₁-C₁₀ alkyl, -SC₁-C₁₀ alkylphenyl, -C₁-C₁₀ alkyl-SH, -SC₁-C₁₀ haloalkyl, halo, -OH, -SH,

-NH₂, -C₁-C₁₀ alkyl-NH₂, -N(C₁-C₁₀ alkyl)(C₁-C₁₀ alkyl), -NH(C₁-C₁₀ alkyl), N(C₁-C₁₀ alkyl) (C₁-C₁₀ alkylphenyl), NH(C₁-C₁₀ alkylphenyl), cyano, nitro, -CO₂H, -C(O)O(C₁-C₁₀ alkyl), -CON(C₁-C₁₀ alkyl)(C₁-C₁₀ alkyl), -CONH(C₁-C₁₀ alkyl), -CONH₂, -NHC(O)(C₁-C₁₀ alkyl), -NHC(O)(phenyl), -N(C₁-C₁₀ alkyl)C(O)(C₁-C₁₀ alkyl), -N(C₁-C₁₀ alkyl)C(O)(phenyl), -C(O)C₁-C₁₀ alkyl, -C(O)C₁-C₁₀ alkylphenyl, -C(O)C₁-C₁₀ haloalkyl, -OC(O)C₁-C₁₀ alkyl, -SO₂(C₁-C₁₀ alkyl), -SO₂(phenyl), -SO₂(C₁-C₁₀ haloalkyl), -SO₂NH₂, -SO₂NH(C₁-C₁₀ alkyl), -SO₂NH(phenyl), -NHSO₂(C₁-C₁₀ alkyl), -NHSO₂(phenyl), and -NHSO₂(C₁-C₁₀ haloalkyl);

each L₁ independently is a linear alkylene of 1 to 70 carbon atoms in length, wherein one or more carbon atoms are optionally replaced with any one or more groups selected from the group consisting of: C(O), NH, O, S, CH=N, S(O)₂, C₂-C₁₀ alkenylene, C₂-C₁₀ alkynylene, C₆-C₁₀ arylene, C₃-C₁₈ heterocyclylene, and C₅-C₁₀ heteroarylene; and wherein L₁ optionally has any one or more substituents selected from the group consisting of: C₁-C₁₀ alkyl, C₆-C₁₀ aryl, C₅-C₁₀ heteroaryl, C₁-C₁₀ haloalkyl, -OC₁-C₁₀ alkyl, -OC₁-C₁₀ alkylphenyl, -C₁-C₁₀ alkyl-OH, -OC₁-C₁₀ haloalkyl, -SC₁-C₁₀ alkyl, -SC₁-C₁₀ alkylphenyl, -C₁-C₁₀ alkyl-SH, -SC₁-C₁₀ haloalkyl, halo, -OH, -SH, -NH₂, -C₁-C₁₀ alkyl-NH₂, -N(C₁-C₁₀ alkyl)(C₁-C₁₀ alkyl), -NH(C₁-C₁₀ alkyl), N(C₁-C₁₀ alkyl) (C₁-C₁₀ alkylphenyl), NH(C₁-C₁₀ alkylphenyl), cyano, nitro, -CO₂H, -C(O)O(C₁-C₁₀ alkyl), -CON(C₁-C₁₀ alkyl)(C₁-C₁₀ alkyl), -CONH(C₁-C₁₀ alkyl), -CONH₂, -NHC(O)(C₁-C₁₀ alkyl), -NHC(O)(phenyl), -N(C₁-C₁₀ alkyl)C(O)(C₁-C₁₀ alkyl), -N(C₁-C₁₀ alkyl)C(O)(phenyl), -C(O)C₁-C₁₀ alkyl, -C(O)C₁-C₁₀ alkylphenyl, -C(O)C₁-C₁₀ haloalkyl, -OC(O)C₁-C₁₀ alkyl, -SO₂(C₁-C₁₀ alkyl), -SO₂(phenyl), -SO₂(C₁-C₁₀ haloalkyl), -SO₂NH₂, -SO₂NH(C₁-C₁₀ alkyl), -SO₂NH(phenyl), -NHSO₂(C₁-C₁₀ alkyl), -NHSO₂(phenyl), and -NHSO₂(C₁-C₁₀ haloalkyl);

~~~~~ represents the site where a group is covalently linked; and M<sub>1</sub> represents a targeting group.

In some embodiments, the present disclosure provides an siRNA capable of inhibiting the expression of PCSK9 gene, wherein the siRNA comprises a sense strand and an antisense strand; each nucleotide in the sense strand and the antisense strand is independently a fluoro modified nucleotide and a non-fluoro modified nucleotide; wherein the sense strand comprises a nucleotide sequence I and the antisense strand comprises a nucleotide sequence II; the nucleotide sequence I and the nucleotide sequence II are at least partly reverse complementary to form a double-stranded region; the fluoro modified nucleotides are located in the nucleotide sequence I and the nucleotide sequence II; and in the direction from 5' terminal to 3' terminal, the nucleotides at positions 7, 8 and 9 of the nucleotide sequence I in the sense strand are fluoro modified nucleotides, and the nucleotides at the rest of positions in the sense strand of the siRNA are non-fluoro modified nucleotides; in the direction from 5' terminal to 3' terminal, the nucleotides at positions 2, 6, 14, and 16 of the nucleotide sequence II in the antisense strand are fluoro modified nucleotides, and the nucleotides at the rest of positions in the antisense strand of the siRNA are non-fluoro modified nucleotides; and the nucleotide sequence I and the nucleotide sequence II are the sequences selected from one of the aforementioned groups i)-vi).

In some embodiments, the present disclosure provides a pharmaceutical composition, comprising the siRNA of the present disclosure, and a pharmaceutically acceptable carrier.

In some embodiments, the present disclosure provides an siRNA conjugate comprising the siRNA of the present disclosure and a conjugation group conjugatively linked to the siRNA.

In some embodiments, the present disclosure provides use of the siRNA, and/or the pharmaceutical composition, and/or the siRNA conjugate of the present disclosure in the manufacture of a medicament for treating and/or preventing diseases or physiological conditions caused by abnormal expression of PCSK9 gene.

5 In some embodiments, the present disclosure provides a method for treating and/or preventing diseases or physiological conditions caused by abnormal expression of PCSK9 gene, comprising administering an effective amount of the siRNA, and/or the pharmaceutical composition, and/or the siRNA conjugate of the present disclosure to a subject in need.

10 In some embodiments, the present disclosure provides a method for inhibiting the expression of PCSK9 gene in hepatocytes, comprising contacting an effective amount of the siRNA, and/or the pharmaceutical composition, and/or the siRNA conjugate of the present disclosure with the hepatocytes.

15 In some embodiments, the present disclosure provides a kit, comprising the siRNA, and/or the pharmaceutical composition, and/or the siRNA conjugate of the present disclosure.

### **BENEFICIAL EFFECTS**

20 The siRNA, the pharmaceutical composition, and the siRNA conjugate of the present disclosure could exhibit good stability, higher inhibitory activity against PCSK9 mRNA, and lower off-target effect and toxicity, and/or can significantly treat and/or prevent diseases or physiological conditions caused by abnormal expression of PCSK9 gene, such as hypercholesterolemia.

25 In some embodiments, the siRNA, the pharmaceutical composition or the siRNA conjugate of the present disclosure exhibits excellent inhibitory activity against the target mRNA in *in vitro* cell experiments. In some embodiments, the siRNA, the pharmaceutical composition or the siRNA conjugate of the present disclosure exhibits an inhibition rate of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% against the target mRNA in hepatocytes.

30 In some embodiments, the siRNA, the pharmaceutical composition or the siRNA conjugate of the present disclosure may have higher stability and/or higher activity *in vivo*. In some embodiments, the siRNA, the pharmaceutical composition or the siRNA conjugate of the present disclosure exhibits an inhibition rate of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% against the expression of the target gene *in vivo*. In some embodiments, the siRNA, the pharmaceutical composition or the siRNA conjugate of the present disclosure exhibits an inhibition rate of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% against the expression of PCSK9 gene *in vivo*. In some embodiments, the siRNA, the pharmaceutical composition or the siRNA conjugate of the present disclosure exhibits an inhibition rate of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% against the expression of PCSK9 gene in livers *in vivo*. In some embodiments, the siRNA, the pharmaceutical composition or the siRNA conjugate of the present disclosure exhibits an inhibition rate of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% against the expression of PCSK9 gene in livers in animal models *in vivo*. In some embodiments, the siRNA, the pharmaceutical composition or the siRNA conjugate of the present disclosure exhibits an inhibition rate of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% against the expression of PCSK9 gene in livers in human subjects *in vivo*. In some embodiments, the siRNA, the pharmaceutical composition or the siRNA conjugate of the present disclosure does not exhibit an obvious off-target effect. Off-target effects can be, for example, inhibition of normal expression of non-target genes. It is believed that if the binding/inhibition of the expression of off-target

genes is less than 50%, 40%, 30%, 20% or 10% compared with the effect on the target gene, the off-target effect is not significant.

In some embodiments, the siRNA of the present disclosure shows higher inhibitory activity in the psiCHECK system, with the IC<sub>50</sub> values ranging from 0.0194 nM to 0.0561 nM for the target sequence. In some embodiments, after a single subcutaneous injection of 9 mg/kg into cynomolgus monkeys, the siRNA conjugate of the present disclosure shows an inhibition rate of up to 56-81% against PCSK9 mRNA in the liver tissue of NHP on day 15 after the administration relative to day 7 before the administration. In some embodiments, the siRNA conjugate of the present disclosure exhibits more significant inhibition against the expression of PCSK9 protein in serum, and shows an inhibition rate of up to more than 90% against the expression of PCSK9 protein in serum on day 14 after administration. In some embodiments, the siRNA conjugate of the present disclosure can significantly inhibit LDL-c and CHO levels in serum, and especially shows an inhibition rate of up to 30%, or even up to 64% against LDL-c during the period from day 14 to day 29 after administration; it also shows a higher inhibition rate, even up to more than 35%, against CHO. In some embodiments, after single administration of 9 mg/kg, the siRNA conjugate of the present disclosure can maintain an inhibition rate of higher than 50% against LDL-c for 11 weeks, and the siRNA conjugate of the present disclosure does not show obvious toxicity in rats or mice. In conclusion, the siRNA conjugate of present disclosure has a rapid onset and long duration of action, can significantly inhibit the expression of target mRNA, can effectively reduce the content of PCSK9 protein in serum, has a strong inhibitory effect on LDL-c and CHO in serum, and have good biological safety.

Therefore, the siRNA, the pharmaceutical composition and the siRNA conjugate of the present disclosure could inhibit the expression of PCSK9 gene, effectively treat and/or prevent diseases or physiological conditions caused by the abnormal expression of PCSK9 gene, and thus show a promising prospect of application.

Additional features and advantages of the present disclosure will be detailedly illustrated in the following part "DETAILED DESCRIPTION OF THE INVENTION".

### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figures 1A-1F show the dose-effect curves fitted according to the relative residual activity of the reporter gene *Renilla* in the psiCHECK system after transfection with different siRNAs (siRNAs 1-6).

Figure 2 shows the normalized PCSK9 protein levels in serum at different time points after a single subcutaneous injection of 9 mg/kg of Conjugate 1, 4 or 7 was given to cynomolgus monkeys.

Figure 3 shows the normalized LDL-c levels in serum at different time points after a single subcutaneous injection of 9 mg/kg of Conjugate 4 or 7 was given to cynomolgus monkeys.

Figure 4 shows the normalized CHO levels in serum at different time points after a single subcutaneous injection of 9 mg/kg of Conjugate 4 or 7 was given to cynomolgus monkeys.

### **DETAILED DESCRIPTION OF THE INVENTION**

The following is the detailed description of the specific embodiments of the present disclosure. It should be understood that the specific embodiments described herein are only used to illustrate and explain the present disclosure and are not intended to limit the present disclosure.

In the present disclosure, PCSK9 mRNA is the mRNA of the sequence as shown in Genbank Accession No. NM\_174936.3. Further, unless otherwise specified, the term “target gene” used in the present disclosure refers to the gene that can transcribe the above PCSK9 mRNA; and the term “target mRNA” refers to the above PCSK9 mRNA.

5

### Definitions

In the context of the present disclosure, unless otherwise specified, C, G, U, A and T represent the base composition of a nucleotide; m represents that the nucleotide adjacent to the left side of the letter m is a methoxy modified nucleotide; f represents that the nucleotide adjacent to the left side of the letter f is a fluoro modified nucleotide; s represents the two nucleotides adjacent to both sides of the letter s are linked by a thiophosphate linkage; P1 represents that the nucleotide adjacent to the right side of P1 is a 5'-phosphate nucleotide or a 5'-phosphate analogue modified nucleotide, VP represents that the nucleotide adjacent to the right side of VP is a vinyl phosphate modified nucleotide; Ps represents that the nucleotide adjacent to the right side of Ps is a thiophosphate modified nucleotide; and P represents that the nucleotide adjacent to the right side of the letter P is a 5'-phosphate nucleotide.

15

In the context of the present disclosure, a “fluoro modified nucleotide” refers to a nucleotide formed by substituting 2'-hydroxy of the ribose group with a fluorine atom. A “non-fluoro modified nucleotide” refers to a nucleotide formed by substituting 2'-hydroxy of the ribose group with a non-fluoro group, or a nucleotide analogue. A “nucleotide analogue” refers to a group that can replace a nucleotide in a nucleic acid, while structurally differs from an adenine ribonucleotide, a guanine ribonucleotide, a cytosine ribonucleotide, a uracil ribonucleotide, or thymine deoxyribonucleotide, such as an isonucleotide, a bridged nucleotide (bridged nucleic acid, BNA) or an acyclic nucleotide. The “methoxy modified nucleotide” refers to a nucleotide formed by substituting 2'-hydroxy of the ribose group with a methoxy group.

25

In the context of the present disclosure, expressions “complementary” and “reverse complementary” can be interchangeably used, and have a well-known meaning in the art, namely, the bases in one strand each are complementarily paired with those in the other strand in a double-stranded nucleic acid molecule. In DNAs, a purine base adenine (A) is always paired with a pyrimidine base thymine (T) (or a uracil (U) in RNAs); and a purine base guanine (G) is always paired with a pyrimidine base cytosine (C). Each base pair comprises a purine and a pyrimidine. While adenines in one strand are always paired with thymines (or uracils) in another strand, and guanines are always paired with cytosines, the two strands are considered as being complementary with each other; and the sequence of a strand may be deduced from the sequence of its complementary strand. Correspondingly, a “mispairing” means that the bases at corresponding positions are not present in a manner of complementary pairing in a double-stranded nucleic acid.

35

In the context of the present disclosure, unless otherwise specified, “basically reverse complementary” means that there are no more than 3 base mispairings between two nucleotide sequences. “Substantially reverse complementary” means that there is no more than 1 base mispairing between two nucleotide sequences. “Completely reverse complementary” means that there is no base mispairing between two nucleotide sequences.

40

In the context of the present disclosure, when a nucleotide sequence has a “nucleotide difference” from another nucleotide sequence, the bases of the nucleotides at the same position therebetween are changed. For example, if a nucleotide base in the latter sequence is A and the nucleotide base at the same position in the former sequence is U, C, G, or T, the two nucleotide sequences are considered as having a nucleotide difference at this

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position. In some embodiments, if a nucleotide at a position is replaced with an abasic nucleotide or a nucleotide analogue, it is also considered that there is a nucleotide difference at the position.

In the context of the present disclosure, particularly in the description of the siRNA, the pharmaceutical composition, or the method for preparing the siRNA conjugate of the present disclosure, unless otherwise specified, the “nucleoside monomer” refers to, according to the type and sequence of the nucleotides in the siRNA or siRNA conjugate to be prepared, unmodified or modified nucleoside phosphoramidite monomer (unmodified or modified RNA phosphoramidites; sometimes RNA phosphoramidites are referred to as nucleoside phosphoramidites) used in a phosphoramidite solid phase synthesis. The phosphoramidite solid phase synthesis is a well-known method for RNA synthesis by those skilled in the art. Nucleoside monomers used in the present disclosure are all commercially available.

In the context of the present disclosure, unless otherwise specified, “conjugation” means that two or more chemical moieties each having specific function are linked to each other via a covalent linkage. Correspondingly, a “conjugate” refers to a compound formed by covalent linkage of individual chemical moieties. Furthermore, a “siRNA conjugate” represents a compound formed by covalently attaching one or more chemical moieties each with specific functions to an siRNA. In the following text, the siRNA conjugate of the present disclosure is sometimes abbreviated as “conjugate”. According to the context of the present disclosure, the siRNA conjugate should be understood as the generic term of many siRNA conjugates, or an siRNA conjugate shown by a chemical formula. In the context of the present disclosure, “conjugation molecules” should be interpreted as specific compounds capable of being conjugated to an siRNA via reactions, thereby finally forming the siRNA conjugate of the present disclosure.

As used herein, “optional” or “optionally” means that the subsequently described event or circumstance may or may not occur, and that the description includes instances in which the event or circumstance occurs and instances in which it does not. For example, “optionally substituted alkyl” encompasses both “alkyl” and “substituted alkyl” as defined below. Those skilled in the art would understand, with respect to any group containing one or more substituents, that such groups are not intended to introduce any substitution or substitution patterns that are sterically impractical, synthetically infeasible and/or inherently unstable.

As used herein, “alkyl” refers to straight chain and branched chain alkyl having the indicated number of carbon atoms, usually from 1 to 20 carbon atoms, for example 1 to 10 carbon atoms, such as 1 to 8 or 1 to 6 carbon atoms. For example, C<sub>1</sub>-C<sub>6</sub> alkyl encompasses both straight and branched chain alkyl of from 1 to 6 carbon atoms. When an alkyl residue having a specific number of carbon atoms is referred to, all branched and straight chain forms having that number of carbon atoms are intended to be encompassed; thus, for example, “butyl” is meant to encompass n-butyl, sec-butyl, isobutyl, and t-butyl; “propyl” includes n-propyl and isopropyl. Alkylene is a subset of alkyl, referring to the same residues as alkyl, but having two attachment points.

As used herein, “alkenyl” refers to an unsaturated branched or straight-chain alkyl group having at least one carbon-carbon double bond obtained by respectively removing one hydrogen molecule from two adjacent carbon atoms of the parent alkyl. The group may be in either the cis or trans configuration of the double bond(s). Typical alkenyl groups include, but are not limited to, ethenyl; propenyl, such as prop-1-en-1-yl, prop-1-en-2-yl, prop-2-en-1-yl (allyl), prop-2-en-2-yl; butenyl, such as but-1-en-1-yl, but-1-en-2-yl, 2-methyl-prop-1-en-1-yl, but-2-en-1-yl, but-2-en-2-yl, buta-1,3-dien-1-yl, buta-1,3-dien-2-yl; and the like. In certain embodiments, an alkenyl group has from 2 to 20 carbon atoms, and in other embodiments, from 2 to 10, 2 to 8, or 2 to 6

carbon atoms. Alkenylene is a subset of alkenyl, referring to the same residues as alkenyl, but having two attachment points.

As used herein, “alkynyl” refers to an unsaturated branched or straight-chain alkyl group having at least one carbon-carbon triple bond obtained by respectively removing two hydrogen molecules from two adjacent carbon atoms of the parent alkyl. Typical alkynyl groups include, but are not limited to, ethynyl; propynyl, such as prop-1-yn-1-yl, prop-2-yn-1-yl; butynyl, such as but-1-yn-1-yl, but-1-yn-3-yl, but-3-yn-1-yl; and the like. In certain embodiments, an alkynyl group has from 2 to 20 carbon atoms, and in other embodiments, from 2 to 10, 2 to 8, or 2 to 6 carbon atoms. Alkynylene is a subset of alkynyl, referring to the same residues as alkynyl, but having two attachment points.

As used herein, “alkoxy” refers to an alkyl group of the indicated number of carbon atoms attached through an oxygen bridge, for example, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, sec-butoxy, tert-butoxy, pentyloxy, 2-pentyloxy, isopentyloxy, neopentyloxy, hexyloxy, 2-hexyloxy, 3-hexyloxy, 3-methylpentyloxy, and the like. Alkoxy group usually has from 1 to 10, 1 to 8, 1 to 6, or 1 to 4 carbon atoms attached through oxygen bridge.

As used herein, “aryl” refers to a radical derived from an aromatic monocyclic or multicyclic hydrocarbon ring system by removing a hydrogen atom from a ring carbon atom. The aromatic monocyclic or multicyclic hydrocarbon ring system contains only a hydrogen atom and 6 to 18 carbon atoms, wherein at least one ring in the ring system is fully unsaturated, i.e., it contains a cyclic, delocalized  $(4n+2)\pi$ -electron system in accordance with the Hückel theory. Aryl groups include, but are not limited to, groups such as phenyl, fluorenyl, and naphthyl. Arylene is a subset of aryl, referring to the same residues as aryl, but having two attachment points.

As used herein, “halo substituent” or “halo” refers to fluoro, chloro, bromo, or iodo, and the term “halogen” includes fluorine, chlorine, bromine, or iodine.

As used herein, “haloalkyl” refers to alkyl as defined above in which the specified number of carbon atoms are substituted with one or more (up to the maximum allowable number) halogen atoms. Examples of haloalkyl include, but are not limited to, trifluoromethyl, difluoromethyl, 2-fluoroethyl, and penta-fluoroethyl.

“Heterocyclyl” refers to a stable 3 to 18 membered non-aromatic ring radical that comprises 2 to 12 carbon atoms and 1 to 6 heteroatoms selected from nitrogen, oxygen or sulfur. Unless stated otherwise in the specification, the heterocyclyl is a monocyclic, bicyclic, tricyclic, or tetracyclic ring system, which may include fused or bridged ring system(s). The heteroatom(s) in the heterocyclyl may be optionally oxidized. One or more nitrogen atoms, if present, are optionally quaternized. The heterocyclyl is partially or fully saturated. The heterocyclyl may be attached to the rest of the molecule through any ring atom. Examples of such heterocyclyl include, but are not limited to, dioxolanyl, thienyl[1,3]dithianyl, decahydroisoquinolyl, imidazolyl, imidazolidinyl, isothiazolidinyl, isoxazolidinyl, morpholinyl, octahydroindolyl, octahydroisoindolyl, 2-oxapiperazinyl, 2-oxapiperidinyl, 2-oxapyrrolidinyl, oxazolidinyl, piperidinyl, piperazinyl, 4-piperidonyl, pyrrolidinyl, pyrazolidinyl, quinuclidinyl, thiazolidinyl, tetrahydrofuryl, trithianyl, tetrahydropyranyl, thiomorpholinyl, thiamorpholinyl, 1-oxo-thiomorpholinyl, and 1,1-dioxo-thiomorpholinyl.

“Heteroaryl” refers to a radical derived from a 3 to 18 membered aromatic ring radical that comprises 2 to 17 carbon atoms and 1 to 6 heteroatoms selected from nitrogen, oxygen or sulfur. As used herein, the heteroaryl may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, wherein at least one ring in the ring system

is fully unsaturated, i.e., it contains a cyclic, delocalized  $(4n+2)\pi$ -electron system in accordance with the Hückel theory. Heteroaryl includes fused or bridged ring system(s). The heteroatom(s) in the heteroaryl is optionally oxidized. One or more nitrogen atoms, if present, are optionally quaternized. The heteroaryl is attached to the rest of the molecule through any ring atom. Examples of heteroaryl include, but are not limited to, azepinyl, acridinyl, benzimidazolyl, benzindolyl, 1,3-benzodioxazolyl, benzofuranyl, benzoxazolyl, benzo[d]thiazolyl, benzothiadiazolyl, benzo[b][1,4]dioxepinyl, benzo[b][1,4]oxazinyl, 1,4-benzodioxanyl, benzonaphthofuranyl, benzooxazolyl, benzodioxolyl, benzodioxinyl, benzopyranyl, benzopyranonyl, benzofuranyl, benzofuranonyl, benzothienyl, benzothieno[3,2-d]pyrimidinyl, benzotriazolyl, benzo[4,6]imidazo[1,2-a]pyridinyl, carbazolyl, cinnolyl, cyclopenta[d]pyrimidinyl, 6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-d]pyrimidinyl, 5,6-dihydrobenzo[h]quinazolyl, 5,6-dihydrobenzo[h]cinnolyl, 6,7-dihydro-5H-benzo[6,7]cyclohepta[1,2-c]pyridazinyl, dibenzofuranyl, dibenzothienyl, furanyl, furanonyl, furo[3,2-c]pyridinyl, 5,6,7,8,9,10 hexahydrocycloocta[d]pyrimidinyl, 5,6,7,8,9,10-hexahydrocyclohepta[d]pyridazinyl, 5,6,7,8,9,10- hexahydrocycloocta[d]pyridinyl, isothiazolyl, imidazolyl, indazolyl, indolyl, isoindolyl, indolyl, isoindolyl, isoquinolyl, indolizyl, isoxazolyl, 5,8-methano-5,6,7,8-tetrahydroquinazolyl, naphthyridinonyl, 1,6-naphthyridinonyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, oxiranyl, 5,6,6a,7,8,9,10,10a-octahydrobenzo[h]quinazolyl, 1-phenyl-1H-pyrrolyl, phenazinyl, phenothiazinyl, phenoxazinyl, phthalazinyl, pteridinyl, purinyl, pyrrolyl, pyrazolyl, pyrazolo[3,4-d]pyrimidinyl, pyridinyl, pyrido[3,2-d]pyrimidinyl, pyrido[3,4-d]pyrimidinyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinazolyl, quinoxalyl, quinolyl, tetrahydroquinolyl, 5,6,7,8-tetrahydroquinazolyl, 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidinyl, 6,7,8,9-tetrahydro-5H-cyclohepta [4,5]thieno[2,3-d]pyrimidinyl, 5,6,7,8-tetrahydropyrido[4,5-c]pyridazinyl, thiazolyl, thiadiazolyl, triazolyl, tetrazolyl, triazinyl, thieno[2,3-d]pyrimidinyl, thieno[3,2-d]pyrimidinyl, thieno[2,3-c]pridinyl and thiophenyl/thienyl.

Various hydroxyl protecting groups may be used in the present disclosure. In general, protecting groups render chemical functional groups inert to specific reaction conditions, and may be appended to and removed from such functional groups in a molecule without substantially damaging the remainder of the molecule. Representative hydroxyl protecting groups are disclosed in Beaucage *et al.*, Tetrahedron 1992, 48, 2223-2311, and also in Greene and Wuts, Protective Groups in Organic Synthesis, Chapter 2, 2d ed, John Wiley & Sons, New York, 1991, which are respectively incorporated herein by reference in their entirety. In some embodiments, the protecting group is stable under basic conditions but may be removed under acidic conditions. In some embodiments, non-exclusive examples of the hydroxyl protecting groups that may be used herein include dimethoxytrityl (DMT), monomethoxytrityl, 9-phenylxanthen-9-yl (Pixyl) and 9-(*p*-methoxyphenyl)xanthen-9-yl (Mox). In some embodiments, non-exclusive examples of the hydroxyl protecting groups that may be used herein comprises Tr (trityl), MMTr (4-methoxytrityl), DMTr (4,4'-dimethoxytrityl), and TMTr (4,4',4''-trimethoxytrityl).

The term “subject”, as used herein, refers to any animal, e.g., a mammal or marsupial. The subject of the present disclosure includes, but are not limited to, human, non-human primate (e.g., rhesus or other types of macaques), mouse, pig, horse, donkey, cow, sheep, rat, and any kind of poultry.

As used herein, “treatment” refers to an approach for obtaining beneficial or desirable results, including but not limited to therapeutic benefit. By “therapeutic benefit” is meant eradication or amelioration of the underlying disorder being treated. Also, a therapeutic benefit is achieved by eradicating or ameliorating one or more physiological symptoms associated with the underlying disorder, thereby observing amelioration in the subject, although the subject may still be afflicted with the underlying disorder.

As used herein, “prevention” refers to an approach for obtaining beneficial or desirable results, including but not limited to a prophylactic benefit. In order to obtain “prophylactic benefit”, the siRNA conjugates or pharmaceutical compositions may be administered to a subject at risk of developing a particular disease, or to a subject reporting one or more physiological symptoms of a disease, even a diagnosis of this disease has not been made.

In one aspect, the present disclosure provides six siRNAs capable of inhibiting the expression of PCSK9 gene.

The siRNA of the present disclosure comprises nucleotide groups as basic structural units. It is well known to those skilled in the art that the nucleotide group comprises a phosphate group, a ribose group and a base. Detailed illustrations of these groups are omitted herein.

The siRNA of the present disclosure comprises a sense strand and an antisense strand. The sense strand and the antisense strand have the same or different length, wherein the sense strand has a length of 19 to 23 nucleotides, and the antisense strand has a length of 19 to 26 nucleotides. In this case, the length ratio of the sense strand and the antisense strand of the siRNA of the present disclosure may be 19/19, 19/20, 19/21, 19/22, 19/23, 19/24, 19/25, 19/26, 20/20, 20/21, 20/22, 20/23, 20/24, 20/25, 20/26, 21/20, 21/21, 21/22, 21/23, 21/24, 21/25, 21/26, 22/20, 22/21, 22/22, 22/23, 22/24, 22/25, 22/26, 23/20, 23/21, 23/22, 23/23, 23/24, 23/25 or 23/26. In some embodiments, the length ratio of the sense strand and the antisense strand of the siRNA is 19/21, 21/23 or 23/25.

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#### **First siRNA**

According to the present disclosure, the siRNA may be a first siRNA.

The first siRNA comprises a sense strand and an antisense strand, and each nucleotide in the first siRNA is independently a modified or unmodified nucleotide, wherein the sense strand comprises a nucleotide sequence I, and the antisense strand comprises a nucleotide sequence II; the nucleotide sequence I and the nucleotide sequence II are at least partly reverse complementary to form a double-stranded region; wherein the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 1 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 2 have an equal length and no more than 3 nucleotide differences:

5'- AAGCAAGCAGACAUUUAUZ<sub>1</sub> -3' (SEQ ID NO: 1);  
5'- Z<sub>2</sub>AUAAAUGUCUGCUUGCUU -3' (SEQ ID NO: 2),

wherein, Z<sub>1</sub> is C and Z<sub>2</sub> is G, and

the nucleotide sequence I comprises a nucleotide Z<sub>3</sub> at the position corresponding to Z<sub>1</sub>; the nucleotide sequence II comprises a nucleotide Z<sub>4</sub> at the position corresponding to Z<sub>2</sub>, wherein Z<sub>4</sub> is the first nucleotide at 5' terminal of the antisense strand;

In the context of the present disclosure, “corresponding position” refers to, the same position in the nucleotide sequence when counting from the same terminal of the nucleotide sequence. For example, the first nucleotide at 3' terminal of the nucleotide sequence I is a nucleotide at the position corresponding to the first nucleotide at 3' terminal of SEQ ID NO: 1.

In some embodiments, the sense strand comprises only the nucleotide sequence I, and the antisense strand comprises only the nucleotide sequence II.

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In some embodiments, the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 1 have no more than 1 nucleotide difference, and/or the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 2 have no more than 1 nucleotide difference.

5 In some embodiments, the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 2 includes a difference at the position  $Z_4$ , wherein  $Z_4$  is selected from A, U or C. In some embodiments, the nucleotide difference is a difference at the position  $Z_4$ , wherein  $Z_4$  is selected from A, U or C. In some embodiments,  $Z_3$  is a nucleotide complementary to  $Z_4$ . The siRNAs with the above-mentioned nucleotide differences have higher inhibitory ability against the target mRNA, and these siRNAs comprising the nucleotide differences are within the protection scope of the present disclosure.

In some embodiments, the nucleotide sequence I and the nucleotide sequence II are basically reverse complementary, substantially reverse complementary, or completely reverse complementary to each other; the “basically reverse complementary” means that there is no more than 3 base mispairings between two nucleotide sequences; the “substantially reverse complementary” means that there is no more than 1 base mispairing between two nucleotide sequences; the “completely reverse complementary” means that there is no mispairing between two nucleotide sequences.

In some embodiments, the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 3, and the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 4:

5'-AAGCAAGCAGACAUUUAU $Z_3$ -3' (SEQ ID NO: 3);

5'- $Z_4$ AUAAAUGUCUGCUUGCUU-3' (SEQ ID NO: 4),

wherein,  $Z_4$  is the first nucleotide at 5' terminal of the antisense strand,  $Z_3$  is selected from A, U, G, or C, and  $Z_4$  is a nucleotide complementary to  $Z_3$ ; in some embodiments,  $Z_3$  is C, and  $Z_4$  is G.

In some embodiments, the sense strand further comprises a nucleotide sequence III, the antisense strand further comprises a nucleotide sequence IV, and the nucleotide sequence III and the nucleotide sequence IV independently of one another have a length of 1 to 4 nucleotides; the nucleotide sequence III and the nucleotide sequence IV have an equal length, and are substantially reverse complementary or completely reverse complementary to each other; the nucleotide sequence III is linked to 5' terminal of the nucleotide sequence I, the nucleotide sequence IV is linked to 3' terminal of the nucleotide sequence II. In some embodiments, the nucleotide sequence IV and a second nucleotide sequence are substantially reverse complementary or completely reverse complementary to each other; the second nucleotide sequence refers to a nucleotide sequence that is adjacent to 5' terminal of the nucleotide sequence shown in SEQ ID NO: 1 of the target mRNA and has the same length as the nucleotide sequence IV.

In some embodiments, the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is C, and the base of the nucleotide sequence IV is G; in this case, the length ratio of the sense strand and the antisense strand thereof is 20/20; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is CC, and the base composition of the nucleotide sequence IV is GG; in this case, the length ratio of the sense strand and the antisense strand thereof is 21/21; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is CCC, and the base composition of the nucleotide sequence IV is GGG; in this case, the length ratio of the sense strand and the antisense strand thereof is 22/22; or, the nucleotide sequence III and the nucleotide

sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is ACCC, and the base composition of the nucleotide sequence IV is GGGU; in this case, the length ratio of the sense strand and the antisense strand thereof is 23/23. In some embodiments, the nucleotide sequence III and the nucleotide sequence IV have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is CC and the base composition of the nucleotide sequence IV is GG; in this case, the length ratio of the sense strand and the antisense strand thereof is 21/21.

In some embodiments, the nucleotide sequence III and the nucleotide sequence IV are completely reverse complementary. Hence, where the base(s) of nucleotide sequence III is(are) provided, the base(s) of nucleotide sequence IV is(are) also determined.

### **Second siRNA**

According to the present disclosure, the siRNA may be a second siRNA.

The second siRNA comprises a sense strand and an antisense strand, and each nucleotide in the second siRNA is independently a modified or unmodified nucleotide; wherein the sense strand comprises a nucleotide sequence I, and the antisense strand comprises a nucleotide sequence II; the nucleotide sequence I and the nucleotide sequence II are at least partly reverse complementary to form a double-stranded region; wherein, the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 61 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 62 have an equal length and no more than 3 nucleotide differences:

5'-UUUGUAGCAUUUUUAUUAZ<sub>5</sub>-3' (SEQ ID NO: 61);

5'-Z<sub>6</sub>UAAUAAAAAUGCUACAAA-3' (SEQ ID NO: 62),

wherein, Z<sub>5</sub> is A and Z<sub>6</sub> is U, and the nucleotide sequence I comprises a nucleotide Z<sub>7</sub> at the position corresponding to Z<sub>5</sub>; the nucleotide sequence II comprises a nucleotide Z<sub>8</sub> at the position corresponding to Z<sub>6</sub>, wherein Z<sub>8</sub> is the first nucleotide at 5' terminal of the antisense strand;

In some embodiments, the sense strand comprises only the nucleotide sequence I, and the antisense strand comprises only the nucleotide sequence II.

In some embodiments, the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 61 have no more than 1 nucleotide difference, and/or the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 62 have no more than 1 nucleotide difference.

In some embodiments, the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 62 includes a difference at the position Z<sub>8</sub>, wherein Z<sub>8</sub> is selected from A, C or G. In some embodiments, the nucleotide difference is a difference at the position Z<sub>8</sub>, wherein Z<sub>8</sub> is selected from A, C or G. In some embodiments, Z<sub>7</sub> is a nucleotide complementary to Z<sub>8</sub>. The siRNAs with the above-mentioned nucleotide differences have higher inhibitory ability against the target mRNA, and these siRNAs comprising the nucleotide differences are within the protection scope of the present disclosure.

In some embodiments, the nucleotide sequence I and the nucleotide sequence II are basically reverse complementary, substantially reverse complementary, or completely reverse complementary to each other.

In some embodiments, the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 63, and the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 64:

5'-UUUGUAGCAUUUUUAUUAZ<sub>7</sub>-3' (SEQ ID NO: 63);

5'-Z<sub>8</sub>UAAUAAAAAUGCUACAAA-3' (SEQ ID NO: 64),

- 5 wherein, Z<sub>8</sub> is the first nucleotide at 5' terminal of the antisense strand, Z<sub>7</sub> is selected from A, U, G, or C, and Z<sub>8</sub> is a nucleotide complementary to Z<sub>7</sub>; in some embodiments, Z<sub>7</sub> is A, and Z<sub>8</sub> is U.

- 10 In some embodiments, the sense strand further comprises a nucleotide sequence III, the antisense strand further comprises a nucleotide sequence IV, and the nucleotide sequence III and the nucleotide sequence IV independently of one another have a length of 1 to 4 nucleotides; the nucleotide sequence III and the nucleotide sequence IV have an equal length, and are substantially reverse complementary or completely reverse complementary to each other; the nucleotide sequence III is linked to 5' terminal of the nucleotide sequence I, the nucleotide sequence IV is linked to 3' terminal of the nucleotide sequence II; the nucleotide sequence IV and a second nucleotide sequence are substantially reverse complementary or completely reverse complementary to each other; the second nucleotide sequence refers to a nucleotide sequence that is adjacent to 5' terminal of the nucleotide sequence shown in SEQ ID NO: 61 of the target mRNA and has the same length as the nucleotide sequence IV.

- 20 In some embodiments, the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is U, and the base of the nucleotide sequence IV is A; in this case, the length ratio of the sense strand and the antisense strand thereof is 20/20; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is GU, and the base composition of the nucleotide sequence IV is AC; in this case, the length ratio of the sense strand and the antisense strand thereof is 21/21; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is GGU, and the base composition of the nucleotide sequence IV is ACC; in this case, the length ratio of the sense strand and the antisense strand thereof is 22/22; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is GGGU and the base composition of the nucleotide sequence IV is ACCC; in this case, the length ratio of the sense strand and the antisense strand thereof is 23/23. In some embodiments, the nucleotide sequence III and the nucleotide sequence IV have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is GU, and the base composition of the nucleotide sequence IV is AC; in this case, the length ratio of the sense strand and the antisense strand thereof is 21/21.

- 40 In some embodiments, the nucleotide sequence III and the nucleotide sequence IV are completely reverse complementary. Hence, where the base(s) of nucleotide sequence III is(are) provided, the base(s) of nucleotide sequence IV is(are) also determined.

### **Third siRNA**

According to the present disclosure, the siRNA may be a third siRNA.

- 45 The third siRNA comprises a sense strand and an antisense strand, and each nucleotide in the third siRNA is independently a modified or unmodified nucleotide; wherein the sense strand comprises a nucleotide sequence I, and the antisense strand comprises a nucleotide sequence II; the nucleotide sequence I and the nucleotide

sequence II are at least partly reverse complementary to form a double-stranded region; wherein, the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 121 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 122 have an equal length and no more than 3 nucleotide differences:

5 5'- GCCUGGAGUUUAUUCGGAZ<sub>9</sub> -3' (SEQ ID NO: 121);

5'- Z<sub>10</sub>UCCGAAUAAACUCCAGGC -3' (SEQ ID NO: 122),

wherein, Z<sub>9</sub> is A and Z<sub>10</sub> is U, and

the nucleotide sequence I comprises a nucleotide Z<sub>11</sub> at the position corresponding to Z<sub>9</sub>; the nucleotide sequence II comprises a nucleotide Z<sub>12</sub> at the position corresponding to Z<sub>10</sub>, wherein Z<sub>12</sub> is the first nucleotide at 5' terminal of the antisense strand;

In some embodiments, the sense strand comprises only the nucleotide sequence I, and the antisense strand comprises only the nucleotide sequence II.

15 In some embodiments, the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 121 have no more than 1 nucleotide difference, and/or the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 122 have no more than 1 nucleotide difference.

20 In some embodiments, the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 122 includes a difference at the position Z<sub>12</sub>, wherein Z<sub>12</sub> is selected from A, C or G. In some embodiments, the nucleotide difference is a difference at the position Z<sub>12</sub>, wherein Z<sub>12</sub> is selected from A, C or G. In some embodiments, Z<sub>11</sub> is a nucleotide complementary to Z<sub>12</sub>. The siRNAs with the above-mentioned nucleotide differences have higher inhibitory ability against the target mRNA, and these siRNAs comprising the nucleotide differences are within the protection scope of the present disclosure.

25 In some embodiments, the nucleotide sequence I and the nucleotide sequence II are basically reverse complementary, substantially reverse complementary, or completely reverse complementary to each other.

30 In some embodiments, the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 123, and the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 124:

5'- GCCUGGAGUUUAUUCGGAZ<sub>11</sub> -3' (SEQ ID NO: 123);

5'- Z<sub>12</sub>UCCGAAUAAACUCCAGGC -3' (SEQ ID NO: 124),

wherein, Z<sub>12</sub> is the first nucleotide at 5' terminal of the antisense strand, Z<sub>11</sub> is selected from A, U, G, or C, and Z<sub>12</sub> is a nucleotide complementary to Z<sub>11</sub>; in some embodiments, Z<sub>11</sub> is A, and Z<sub>12</sub> is U.

35 In some embodiments, the sense strand further comprises a nucleotide sequence III, the antisense strand further comprises a nucleotide sequence IV, and the nucleotide sequence III and the nucleotide sequence IV independently of one another have a length of 1 to 4 nucleotides; the nucleotide sequence III and the nucleotide sequence IV have an equal length, and are substantially reverse complementary or completely reverse complementary to each other; the nucleotide sequence III is linked to 5' terminal of the nucleotide sequence I, the nucleotide sequence IV is linked to 3' terminal of the nucleotide sequence II; the nucleotide sequence IV and a second nucleotide sequence are substantially reverse complementary or completely reverse complementary to each other; the second nucleotide sequence refers to a nucleotide sequence that is adjacent to 5' terminal of the nucleotide sequence shown in SEQ ID NO: 121 of the target mRNA and has the same length as the nucleotide sequence IV.

In some embodiments, the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is G, and the base of the nucleotide sequence IV is C; in this case, the length ratio of the sense strand and the antisense strand thereof is 20/20; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the **base composition** of the nucleotide sequence III is AG, and the **base composition** of the nucleotide sequence IV is CU; in this case, the length ratio of the sense strand and the antisense strand thereof is 21/21; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the **base composition** of the nucleotide sequence III is UAG, and the **base composition** of the nucleotide sequence IV is CUA; in this case, the length ratio of the sense strand and the antisense strand thereof is 22/22; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the **base composition** of the nucleotide sequence III is AUAG, and the **base composition** of the nucleotide sequence IV is CUAU; in this case, the length ratio of the sense strand and the antisense strand thereof is 23/23. In some embodiments, the nucleotide sequence III and the nucleotide sequence IV have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AG, and the base composition of the nucleotide sequence IV is CU; in this case, the length ratio of the sense strand and the antisense strand thereof is 21/21.

In some embodiments, the nucleotide sequence III and the nucleotide sequence IV are completely reverse complementary. Hence, where the base(s) of nucleotide sequence III is(are) provided, the base(s) of nucleotide sequence IV is(are) also determined.

#### **Fourth siRNA**

According to the present disclosure, the siRNA may be a fourth siRNA.

The fourth siRNA comprises a sense strand and an antisense strand, and each nucleotide in the fourth siRNA is independently a modified or unmodified nucleotide; wherein the sense strand comprises a nucleotide sequence I, and the antisense strand comprises a nucleotide sequence II; the nucleotide sequence I and the nucleotide sequence II are at least partly reverse complementary to form a double-stranded region; wherein, the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 181 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 182 have an equal length and no more than 3 nucleotide differences:

5'- CUGUUUUGCUUUUGUAACZ<sub>13</sub> -3' (SEQ ID NO: 181);

5'- Z<sub>14</sub>GUUACAAAAGCAAAACAG -3' (SEQ ID NO: 182),

wherein, Z<sub>13</sub> is U and Z<sub>14</sub> is A, and the nucleotide sequence I comprises a nucleotide Z<sub>15</sub> at the position corresponding to Z<sub>13</sub>, the nucleotide sequence II comprises a nucleotide Z<sub>16</sub> at the position corresponding to Z<sub>14</sub>, wherein Z<sub>16</sub> is the first nucleotide at 5' terminal of the antisense strand.

In some embodiments, the sense strand comprises only the nucleotide sequence I, and the antisense strand comprises only the nucleotide sequence II.

In some embodiments, the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 181 have no more than 1 nucleotide difference, and/or the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 182 have no more than 1 nucleotide difference.

In some embodiments, the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 182 includes a difference at the position  $Z_{16}$ , wherein  $Z_{16}$  is selected from U, C or G. In some embodiments, the nucleotide difference is a difference at the position  $Z_{16}$ , wherein  $Z_{16}$  is selected from U, C or G. In some embodiments,  $Z_{15}$  is a nucleotide complementary to  $Z_{16}$ . The siRNAs with the above-mentioned nucleotide differences have higher inhibitory ability against the target mRNA, and these siRNAs comprising the nucleotide differences are within the protection scope of the present disclosure.

In some embodiments, the nucleotide sequence I and the nucleotide sequence II are basically reverse complementary, substantially reverse complementary, or completely reverse complementary to each other.

In some embodiments, the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 183, and the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 184:

5'- CUGUUUUGCUUUUGUAAAC $Z_{15}$  -3' (SEQ ID NO: 183);  
 5'-  $Z_{16}$ GUUACAAAAGCAAAACAG -3' (SEQ ID NO: 184),  
 wherein,  $Z_{16}$  is the first nucleotide at 5' terminal of the antisense strand,  $Z_{15}$  is selected from A, U, G, or C, and  $Z_{16}$  is a nucleotide complementary to  $Z_{15}$ ; in some embodiments,  $Z_{15}$  is U, and  $Z_{16}$  is A.

In some embodiments, the sense strand further comprises a nucleotide sequence III, the antisense strand further comprises a nucleotide sequence IV, and the nucleotide sequence III and the nucleotide sequence IV independently of one another have a length of 1 to 4 nucleotides; the nucleotide sequence III and the nucleotide sequence IV have an equal length, and are substantially reverse complementary or completely reverse complementary to each other; the nucleotide sequence III is linked to 5' terminal of the nucleotide sequence I, the nucleotide sequence IV is linked to 3' terminal of the nucleotide sequence II; the nucleotide sequence IV and a second nucleotide sequence are substantially reverse complementary or completely reverse complementary to each other; the second nucleotide sequence refers to a nucleotide sequence that is adjacent to 5' terminal of the nucleotide sequence shown in SEQ ID NO: 181 of the target mRNA and has the same length as the nucleotide sequence IV.

In some embodiments, the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is C, and the base of the nucleotide sequence IV is G; in this case, the length ratio of the sense strand and the antisense strand thereof is 20/20; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AC, and the base composition of the nucleotide sequence IV is GU; in this case, the length ratio of the sense strand and the antisense strand thereof is 21/21; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is GAC, and the base composition of the nucleotide sequence IV is GUC; in this case, the length ratio of the sense strand and the antisense strand thereof is 22/22; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AGAC, and the base composition of the nucleotide sequence IV is GUCU; in this case, the length ratio of the sense strand and the antisense strand thereof is 23/23. In some embodiments, the nucleotide sequence III and the nucleotide sequence IV have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AC, and the base composition of the nucleotide sequence IV is GU; in this case, the length ratio of the sense strand and the antisense strand thereof is 21/21.

In some embodiments, the nucleotide sequence III and the nucleotide sequence IV are completely reverse complementary. Hence, where the base(s) of nucleotide sequence III is(are) provided, the base(s) of nucleotide sequence IV is(are) also determined.

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#### **Fifth siRNA**

According to the present disclosure, the siRNA may be a fifth siRNA.

The fifth siRNA comprises a sense strand and an antisense strand, and each nucleotide in the fifth siRNA is independently a modified or unmodified nucleotide; wherein the sense strand comprises a nucleotide sequence I, and the antisense strand comprises a nucleotide sequence II; the nucleotide sequence I and the nucleotide sequence II are at least partly reverse complementary to form a double-stranded region; wherein, the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 241 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 242 have an equal length and no more than 3 nucleotide differences:

5'-GGUUUUGUAGCAUUUUUAZ<sub>17</sub>-3' (SEQ ID NO: 241);

5'-Z<sub>18</sub>UAAAAAUGCUACAAAACC-3' (SEQ ID NO: 242),

wherein, Z<sub>17</sub> is U and Z<sub>18</sub> is A, and

the nucleotide sequence I comprises a nucleotide Z<sub>19</sub> at the position corresponding to Z<sub>17</sub>, the nucleotide sequence II comprises a nucleotide Z<sub>20</sub> at the position corresponding to Z<sub>18</sub>, wherein Z<sub>20</sub> is the first nucleotide at 5' terminal of the antisense strand.

25

In some embodiments, the sense strand comprises only the nucleotide sequence I, and the antisense strand comprises only the nucleotide sequence II.

In some embodiments, the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 241 have no more than 1 nucleotide difference, and/or the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 242 have no more than 1 nucleotide difference.

In some embodiments, the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 242 includes a difference at the position Z<sub>20</sub>, wherein Z<sub>20</sub> is selected from U, C or G. In some embodiments, the nucleotide difference is a difference at the position Z<sub>20</sub>, wherein Z<sub>20</sub> is selected from U, C or G. In some embodiments, Z<sub>19</sub> is a nucleotide complementary to Z<sub>20</sub>. The siRNAs with the above-mentioned nucleotide differences have higher inhibitory ability against the target mRNA, and these siRNAs comprising the nucleotide differences are within the protection scope of the present disclosure.

In some embodiments, the nucleotide sequence I and the nucleotide sequence II are basically reverse complementary, substantially reverse complementary, or completely reverse complementary to each other.

In some embodiments, the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 243, and the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 244:

5'-GGUUUUGUAGCAUUUUUAZ<sub>19</sub>-3' (SEQ ID NO: 243);

5'-Z<sub>20</sub>UAAAAAUGCUACAAAACC-3' (SEQ ID NO: 244),

wherein, Z<sub>20</sub> is the first nucleotide at 5' terminal of the antisense strand, Z<sub>19</sub> is selected from A, U, G, or C, and

Z<sub>20</sub> is a nucleotide complementary to Z<sub>19</sub>; in some embodiments, Z<sub>19</sub> is U, and Z<sub>20</sub> is A.

In some embodiments, the sense strand further comprises a nucleotide sequence III, the antisense strand further comprises a nucleotide sequence IV, and the nucleotide sequence III and the nucleotide sequence IV independently of one another have a length of 1 to 4 nucleotides; the nucleotide sequence III and the nucleotide sequence IV have an equal length, and are substantially reverse complementary or completely reverse complementary to each other; the nucleotide sequence III is linked to 5' terminal of the nucleotide sequence I, the nucleotide sequence IV is linked to 3' terminal of the nucleotide sequence II; the nucleotide sequence IV and a second nucleotide sequence are substantially reverse complementary or completely reverse complementary to each other; the second nucleotide sequence refers to a nucleotide sequence that is adjacent to 5' terminal of the nucleotide sequence shown in SEQ ID NO: 241 of the target mRNA and has the same length as the nucleotide sequence IV.

In some embodiments, the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is G, and the base of the nucleotide sequence IV is C; in this case, the length ratio of the sense strand and the antisense strand thereof is 20/20; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UG, and the base composition of the nucleotide sequence IV is CA; in this case, the length ratio of the sense strand and the antisense strand thereof is 21/21; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is CUG, and the base composition of the nucleotide sequence IV is CAG; in this case, the length ratio of the sense strand and the antisense strand thereof is 22/22; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UCUG, and the base composition of the nucleotide sequence IV is CAGA; in this case, the length ratio of the sense strand and the antisense strand thereof is 23/23. In some embodiments, the nucleotide sequence III and the nucleotide sequence IV have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UG, and the base composition of the nucleotide sequence IV is CA; in this case, the length ratio of the sense strand and the antisense strand thereof is 21/21.

In some embodiments, the nucleotide sequence III and the nucleotide sequence IV are completely reverse complementary. Hence, where the base(s) of nucleotide sequence III is(are) provided, the base(s) of nucleotide sequence IV is(are) also determined.

### **Sixth siRNA**

According to the present disclosure, the siRNA may be a sixth siRNA.

The sixth siRNA comprises a sense strand and an antisense strand, and each nucleotide in the sixth siRNA is independently a modified or unmodified nucleotide; wherein the sense strand comprises a nucleotide sequence I, and the antisense strand comprises a nucleotide sequence II; the nucleotide sequence I and the nucleotide sequence II are at least partly reverse complementary to form a double-stranded region; wherein, the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 301 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 302 have an equal length and no more than 3 nucleotide differences:

5'- GUGACUUUUUAAAAUAAAZ<sub>21</sub> -3' (SEQ ID NO: 301);  
 5'- Z<sub>22</sub>UUUAUUUUAAAAAGUCAC -3' (SEQ ID NO: 302),  
 wherein, Z<sub>21</sub> is A and Z<sub>22</sub> is U, and

the nucleotide sequence I comprises a nucleotide  $Z_{23}$  at the position corresponding to  $Z_{21}$ , the nucleotide sequence II comprises a nucleotide  $Z_{24}$  at the position corresponding to  $Z_{22}$ , wherein  $Z_{24}$  is the first nucleotide at 5' terminal of the antisense strand.

- 5 In some embodiments, the sense strand comprises only the nucleotide sequence I, and the antisense strand comprises only the nucleotide sequence II.

In some embodiments, the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 301 have no more than 1 nucleotide difference, and/or the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 302 have no more than 1 nucleotide difference.

In some embodiments, the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 302 includes a difference at the position  $Z_{24}$ , wherein  $Z_{24}$  is selected from A, C or G. In some embodiments, the nucleotide difference is a difference at the position  $Z_{24}$ , wherein  $Z_{24}$  is selected from A, C or G. In some embodiments,  $Z_{23}$  is a nucleotide complementary to  $Z_{24}$ . The siRNAs with the above-mentioned nucleotide differences have higher inhibitory ability against the target mRNA, and these siRNAs comprising the nucleotide differences are within the protection scope of the present disclosure.

In some embodiments, the nucleotide sequence I and the nucleotide sequence II are basically reverse complementary, substantially reverse complementary, or completely reverse complementary to each other.

In some embodiments, the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 303, and the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 304:

5' - GUGACUUUUUAAAAUAAAZ<sub>23</sub> -3' (SEQ ID NO: 303);  
 5' - Z<sub>24</sub>UUUAUUUUAAAAAGUCAC -3' (SEQ ID NO: 304),  
 wherein,  $Z_{24}$  is the first nucleotide at 5' terminal of the antisense strand,  $Z_{23}$  is selected from A, U, G, or C, and  $Z_{24}$  is a nucleotide complementary to  $Z_{23}$ ; in some embodiments,  $Z_{23}$  is A, and  $Z_{24}$  is U.

In some embodiments, the sense strand further comprises a nucleotide sequence III, the antisense strand further comprises a nucleotide sequence IV, and the nucleotide sequence III and the nucleotide sequence IV independently of one another have a length of 1 to 4 nucleotides; the nucleotide sequence III and the nucleotide sequence IV have an equal length, and are substantially reverse complementary or completely reverse complementary to each other; the nucleotide sequence III is linked to 5' terminal of the nucleotide sequence I, the nucleotide sequence IV is linked to 3' terminal of the nucleotide sequence II; the nucleotide sequence IV and a second nucleotide sequence are substantially reverse complementary or completely reverse complementary to each other; the second nucleotide sequence refers to a nucleotide sequence that is adjacent to 5' terminal of the nucleotide sequence shown in SEQ ID NO: 301 of the target mRNA and has the same length as the nucleotide sequence IV.

In some embodiments, the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is G, and the base of the nucleotide sequence IV is C; in this case, the length ratio of the sense strand and the antisense strand thereof is 20/20; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UG, and the base composition of the nucleotide sequence IV is CA; in this case, the length ratio of the sense strand and the antisense strand thereof is 21/21; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 3

nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AUG, and the base composition of the nucleotide sequence IV is CAU; in this case, the length ratio of the sense strand and the antisense strand thereof is 22/22; or, the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UAUG, and the base composition of the nucleotide sequence IV is CAUA; in this case, the length ratio of the sense strand and the antisense strand thereof is 23/23. In some embodiments, the nucleotide sequence III and the nucleotide sequence IV have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UG, and the base composition of the nucleotide sequence IV is CA; in this case, the length ratio of the sense strand and the antisense strand thereof is 21/21.

In some embodiments, the nucleotide sequence III and the nucleotide sequence IV are completely reverse complementary. Hence, where the base(s) of nucleotide sequence III is(are) provided, the base(s) of nucleotide sequence IV is(are) also determined.

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#### **Overhang terminal and modification of siRNA**

The following description regarding the nucleotide sequence V, the nucleic acid sequence, or the nucleotide modification and the modified sequence of the siRNA is applicable to any one of the first siRNA to the sixth siRNA. Namely, unless stated otherwise, the following description of the siRNA should be regarded as the description of each of the first, second, third, fourth, fifth and sixth siRNAs. For example, if no particular siRNA is specifically indicated, "the siRNA further comprises a nucleotide sequence V" means that "the first siRNA, the second siRNA, the third siRNA, the fourth siRNA, the fifth siRNA and the sixth siRNA further comprise a nucleotide sequence V".

In some embodiments, the sense strand and the antisense strand have the same or different length, and the antisense strand further comprises a nucleotide sequence V. The nucleotide sequence V has a length of 1 to 3 nucleotides and is linked to 3' terminal of the antisense strand, thereby forming a 3' overhang terminal of the antisense strand. In this case, the length ratio of the sense strand and the antisense strand of the siRNA of the present disclosure may be 19/20, 19/21, 19/22, 20/21, 20/22, 20/23, 21/22, 21/23, 21/24, 22/23, 22/24, 22/25, 23/24, 23/25, or 23/26. In some embodiments, the nucleotide sequence V has a length of 2 nucleotides. Thus, the length ratio of the sense strand and the antisense strand of the siRNA of the present disclosure may be 19/21, 21/23 or 23/25.

Each nucleotide in the nucleotide sequence V may be any nucleotide. In order to facilitate the synthesis and to save synthesis cost, the nucleotide sequence V is 2 consecutive thymine deoxyribonucleotides (dTdT) or 2 consecutive uracil ribonucleotides (UU); in order to enhance the affinity between the antisense strand of the siRNA and the target mRNA, the nucleotide sequence V is complementary to the nucleotides at the corresponding positions of the target mRNA. Thus, in some embodiments, the length ratio of the sense strand and the antisense strand of the siRNA of the present disclosure is 19/21 or 21/23. In this case, the siRNA of the present disclosure exhibits better mRNA silencing activity.

The nucleotides at the corresponding positions of the target mRNA refer to the nucleotides or nucleotide sequence adjacent to 5' terminal of a third nucleotide sequence of the target mRNA. This third nucleotide sequence refers to the segment of the nucleotide sequence which is substantially reverse complementary or completely reverse complementary with the nucleotide sequence II, or is substantially reverse complementary

or completely reverse complementary with the nucleotide sequence consisted of the nucleotide sequence II and the nucleotide sequence IV.

In some embodiments, for the first siRNA, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 5, and the antisense strand comprises the nucleotide sequence as shown by SEQ ID NO: 6:

5'- AAGCAAGCAGACAUUUAU<sub>Z3</sub> -3' (SEQ ID NO: 5);  
5'- Z<sub>4</sub>AUAAAUGUCUGCUUGCUUGG -3' (SEQ ID NO: 6);

Alternatively, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 7, and the antisense strand comprises the nucleotide sequence as shown by SEQ ID NO: 8:

5'- CCAAGCAAGCAGACAUUUAU<sub>Z3</sub> -3' (SEQ ID NO: 7);  
5'- Z<sub>4</sub>AUAAAUGUCUGCUUGCUUGGGU -3' (SEQ ID NO: 8);

wherein, Z<sub>4</sub> is the first nucleotide at 5' terminal of the antisense strand; Z<sub>3</sub> is selected from A, U, G or C, and

Z<sub>4</sub> is a nucleotide complementary to Z<sub>3</sub>.

In some embodiments, for the second siRNA, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 65, and the antisense strand comprises the nucleotide sequence as shown by SEQ ID NO: 66:

5'- UUUGUAGCAUUUUUAUUAZ<sub>7</sub> -3' (SEQ ID NO: 65);  
5'- Z<sub>8</sub>UAAUAAAAAUGCUACAAAAC -3' (SEQ ID NO: 66),

Alternatively, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 67, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 68:

5'- GUUUUGUAGCAUUUUUAUUAZ<sub>7</sub> -3' (SEQ ID NO: 67);  
5'- Z<sub>8</sub>UAAUAAAAAUGCUACAAAACCC -3' (SEQ ID NO: 68),

wherein, Z<sub>8</sub> is the first nucleotide at 5' terminal of the antisense strand; Z<sub>7</sub> is selected from A, U, G or C, and Z<sub>8</sub> is a nucleotide complementary to Z<sub>7</sub>.

In some embodiments, for the third siRNA, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 125, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 126:

5'- GCCUGGAGUUUAUUCGGAZ<sub>11</sub> -3' (SEQ ID NO: 125);  
5'-Z<sub>12</sub>UCCGAAUAAACUCCAGGCCU -3' (SEQ ID NO: 126),

Alternatively, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 127, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 128:

5'- AGGCCUGGAGUUUAUUCGGAZ<sub>11</sub> -3' (SEQ ID NO: 127);  
5'-Z<sub>12</sub>UCCGAAUAAACUCCAGGCCUAU -3' (SEQ ID NO: 128),

wherein, Z<sub>12</sub> is the first nucleotide at 5' terminal of the antisense strand; Z<sub>11</sub> is selected from A, U, G or C, and Z<sub>12</sub> is a nucleotide complementary to Z<sub>11</sub>.

In some embodiments, for the fourth siRNA, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 185, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 186:

5'- CUGUUUUGCUUUUGUAACZ<sub>15</sub> -3' (SEQ ID NO: 185);  
5'-Z<sub>16</sub>GUUACAAAAGCAAAACAGGU -3' (SEQ ID NO: 186),

Alternatively, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 187, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 188:

5'-ACCUGUUUUGCUUUUGUAACZ<sub>15</sub>-3' (SEQ ID NO: 187);

5'-Z<sub>16</sub>GUUACAAAAGCAAAACAGGUCU-3' (SEQ ID NO: 188),

wherein, Z<sub>16</sub> is the first nucleotide at 5' terminal of the antisense strand; Z<sub>15</sub> is selected from A, U, G or C, and Z<sub>16</sub> is a nucleotide complementary to Z<sub>15</sub>.

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In some embodiments, for the fifth siRNA, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 245, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 246:

5'-GGUUUUGUAGCAUUUUUAZ<sub>19</sub>-3' (SEQ ID NO: 245);

10 5'-Z<sub>20</sub>UAAAAAUGCUACAAAACCCA-3' (SEQ ID NO: 246),

Alternatively, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 247, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 248:

5'-UGGGUUUUGUAGCAUUUUUAZ<sub>19</sub>-3' (SEQ ID NO: 247);

5'-Z<sub>20</sub>UAAAAAUGCUACAAAACCCAGA-3' (SEQ ID NO: 248),

15 wherein, Z<sub>20</sub> is the first nucleotide at 5' terminal of the antisense strand; Z<sub>19</sub> is selected from A, U, G or C, and Z<sub>20</sub> is a nucleotide complementary to Z<sub>19</sub>.

In some embodiments, for the sixth siRNA, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 305, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 306:

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5'-GUGACUUUUUAAAAUAAAZ<sub>23</sub>-3' (SEQ ID NO: 305);

5'-Z<sub>24</sub>UUUAAUUUAAAAAGUCACCA-3' (SEQ ID NO: 306),

Alternatively, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 307, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 308:

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5'-UGGUGACUUUUUAAAAUAAAZ<sub>23</sub>-3' (SEQ ID NO: 307);

5'-Z<sub>24</sub>UUUAAUUUAAAAAGUCACCAUA-3' (SEQ ID NO: 308),

wherein, Z<sub>24</sub> is the first nucleotide at 5' terminal of the antisense strand; Z<sub>23</sub> is selected from A, U, G or C, and Z<sub>24</sub> is a nucleotide complementary to Z<sub>23</sub>.

30 In some embodiments, the siRNA of the present disclosure is siPCSKa1, siPCSKa2, siPCSKb1, siPCSKb2, siPCSKc1, siPCSKc2, siPCSKd1, siPCSKd2, siPCSKe1, siPCSKe2, siPCSKf1 or siPCSKf2 listed in Tables 1a-1f.

35 As mentioned above, in the siRNA of the present disclosure, each nucleotide is independently a modified or unmodified nucleotide. In some embodiments, the nucleotide in the siRNA of the present disclosure is an unmodified nucleotide; in some embodiments, in the siRNA of the present disclosure, some or all of the nucleotides are modified nucleotides. These modifications on the nucleotide groups would not lead to significant decrease or loss of the functions for inhibiting the expression of PCSK9 gene of the siRNA of the present disclosure.

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In some embodiments, the siRNA of the present disclosure comprises at least 1 modified nucleotide. In the context of the present disclosure, the term "modified nucleotide" used refers to a nucleotide formed by substituting 2'-hydroxy of the ribose group with other groups, or nucleotide analogue, or a nucleotide with a modified base. The modified nucleotide would not lead to significant impairment or loss of the functions for inhibiting gene expression of the siRNA conjugate. For example, the modified nucleotides disclosed in *Watts*,

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*J.K., G. F. Deleavey and M. J. Damha, Chemically Modified siRNA: tools and applications. Drug Discov Today, 2008.13(19-20): p.842-55 may be selected.*

5 In some embodiments, at least one nucleotide in the sense strand or the antisense strand of the siRNA of the present disclosure is a modified nucleotide, and/or at least one phosphate group is a phosphate group with modified group(s). In other words, at least a portion of the phosphate and/or ribose groups in the phosphate-ribose backbone of at least one single strand in the sense strand and the antisense strand are phosphate groups with modified groups and/or ribose groups with modified groups.

10 In some embodiments, all the nucleotides in the sense strand and/or the antisense strand are modified nucleotides. In some embodiments, each nucleotide in the sense strand and antisense strand of the siRNA of the present disclosure is independently a fluoro modified nucleotide or a non-fluoro modified nucleotide.

15 The inventors of the present disclosure surprisingly found that the siRNA of the present disclosure achieves high balance between plasma stability and gene silencing efficiency in animal experiments.

20 In some embodiments, the fluoro modified nucleotides are located in the nucleotide sequence I and the nucleotide sequence II; and in the direction from 5' terminal to 3' terminal, the nucleotides at least at positions 7, 8 and 9 of the nucleotide sequence I are fluoro modified nucleotides; and in the direction from 5' terminal to 3' terminal, the nucleotides at least at positions 2, 6, 14, and 16 of the nucleotide sequence II are fluoro modified nucleotides.

25 In some embodiments, the fluoro modified nucleotides are located in the nucleotide sequence I and the nucleotide sequence II; and the nucleotide sequence I comprises no more than 5 fluoro modified nucleotides; and in the direction from 5' terminal to 3' terminal, the nucleotides at positions 7, 8 and 9 of the nucleotide sequence I are fluoro modified nucleotides; the nucleotide sequence II comprises no more than 7 fluoro modified nucleotides; and the nucleotides at positions 2, 6, 14, and 16 of the nucleotide sequence II are fluoro modified nucleotides.

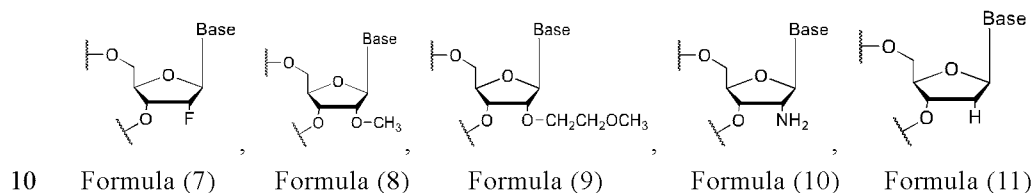
30 In some embodiments, in the direction from 5' terminal to 3' terminal, the nucleotides at positions 7, 8 and 9 or at positions 5, 7, 8 and 9 of the nucleotide sequence I in the sense strand are fluoro modified nucleotides, and the nucleotides at the other positions in the sense strand are non-fluoro modified nucleotides; in the direction from 5' terminal to 3' terminal, the nucleotides at positions 2, 6, 14, and 16 or at positions 2, 6, 8, 9, 14, and 16 of the nucleotide sequence II in the antisense strand are fluoro modified nucleotides, and the  
35 nucleotides at the other positions in the antisense strand are non-fluoro modified nucleotides.

40 In the context of the present disclosure, a "fluoro modified nucleotide" refers to a nucleotide formed by substituting 2'-hydroxy of the ribose group with a fluorine atom, which has a structure as shown by the following Formula (7). A "non-fluoro modified nucleotide" refers to a nucleotide formed by substituting 2'-hydroxy of the ribose group with a non-fluoro group, or a nucleotide analogue. In some embodiments, each non-fluoro modified nucleotide is independently selected from a nucleotide formed by substituting 2'-hydroxy of the ribose group of a nucleotide with a non-fluoro group, or a nucleotide analogue.

45 The nucleotides formed by replacing 2'-hydroxy of the ribose group with a non-fluoro group are well-known in the art, and can be one selected from the group consisting of 2'-alkoxy modified nucleotides, 2'-substituted

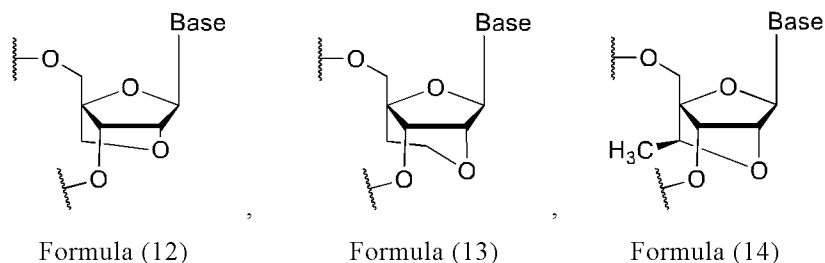
alkoxy modified nucleotides, 2'-alkyl modified nucleotides, 2'-substituted alkyl modified nucleotides, 2'-amino modified nucleotides, 2'-substituted amino modified nucleotides, and 2'-deoxy nucleotides.

- In some embodiments, the 2'-alkoxy modified nucleotide is a 2'-methoxy (2'-OMe) modified nucleotide, as shown by Formula (8). In some embodiments, the 2'-substituted alkoxy modified nucleotide is for example a 2'-O-methoxyethyl (2'-MOE) modified nucleotide, as shown by Formula (9). In some embodiments, the 2'-amino (2'-NH<sub>2</sub>) modified nucleotide is as shown by Formula (10). In some embodiments, the 2'-deoxy nucleotide (DNA) is as shown by Formula (11).

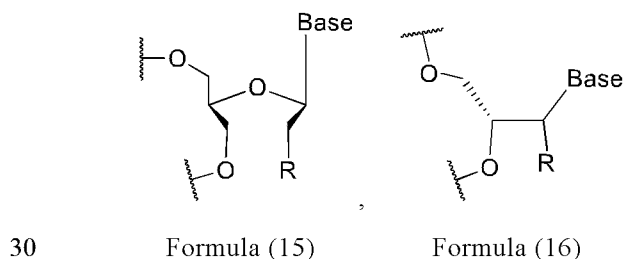


- A nucleotide analogue refers to a group that can replace a nucleotide in a nucleic acid, while structurally differs from an adenine ribonucleotide, a guanine ribonucleotide, a cytosine ribonucleotide, a uracil ribonucleotide, or thymine deoxyribonucleotide. In some embodiments, the nucleotide analogue may be an isonucleotide, a bridged nucleotide or an acyclic nucleotide.

- A BNA nucleotide (bridged nucleic acid, BNA) is a nucleotide that is constrained or inaccessible. BNA can contain a 5-, 6-membered or a 7-membered ring bridged structure with a “fixed” C3'-endo sugar puckering. The bridge is typically incorporated at the 2'- and 4'-positions of the ribose to afford a 2',4'-BNA nucleotide. In some embodiments, BNA may be LNA, ENA, cET BNA and so on, which are shown by Formulae (12), (13) and (14), respectively.

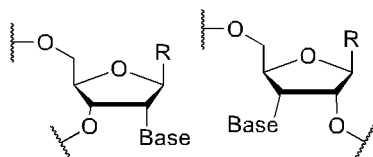


- An acyclic nucleotide refers to a class of nucleotides in which the sugar ring is opened. In some embodiments, the acyclic nucleotide is an unlocked nucleic acid (UNA) or a glycerol nucleic acid (GNA), which are as shown by Formulae (15) and (16), respectively.



In the above Formulae (15) and (16), R is selected from H, OH or alkoxy (O-alkyl).

An isonucleotide is a compound formed by changing the position of the base on the ribose ring in the nucleotide. In some embodiments, the isonucleotide is a compound formed by transposing the base from 1'-position to 2'-position or 3'-position on the ribose ring, as shown by Formula (17) or (18), respectively.



5                      Formula (17)                      Formula (18)

In the above compounds of Formulae (17)-(18), "Base" represents a base, such as A, U, G, C, or T; R is selected from H, OH, F, or the above non-fluoro group.

10        In some embodiments, a nucleotide analogue is one selected from the group consisting of isonucleotide, LNA, ENA, cET, UNA, and GNA. In some embodiments, each non-fluoro modified nucleotide is a methoxy modified nucleotide. In the context of the present disclosure, the methoxy modified nucleotide refers to a nucleotide formed by substituting 2'-hydroxy of the ribose group with a methoxy group.

15        In the context of the disclosure, a "fluoro modified nucleotide", a "2'-fluoro modified nucleotide", a "nucleotide in which 2'-hydroxy of a ribose group is substituted with a fluorine atom", and a "nucleotide with 2'-fluororibosyl" have the same meaning, referring to a compound in which 2'-hydroxy of the nucleotide is substituted with a fluorine atom, which has a structure as shown by Formula (7). A "methoxy modified nucleotide", a "2'-methoxy modified nucleotide", a "nucleotide in which 2'-hydroxy of a ribose group is substituted with a methoxy" and a "nucleotide with 2'-methoxyribosyl" have the same meaning, referring to a compound in which 2'-hydroxy of the ribose group in the nucleotide is substituted with a methoxy, which has a structure as shown by Formula (8).

20

In some embodiments, the siRNA of the present disclosure is an siRNA with the following modifications: in the direction from 5' terminal to 3' terminal, the nucleotides at positions 7, 8 and 9 or at positions 5, 7, 8 and 9 of the nucleotide sequence I in the sense strand are fluoro modified nucleotides, and the nucleotides at the other positions in the sense strand are methoxy modified nucleotides; the nucleotides at positions 2, 6, 14, and 16 or at positions 2, 6, 8, 9, 14, and 16 of the nucleotide sequence II in the antisense strand are fluoro modified nucleotides, and the nucleotides at the other positions in the antisense strand are methoxy modified nucleotides.

25

30        In some embodiments, the siRNA of the present disclosure is an siRNA with the following modifications: in the direction from 5' terminal to 3' terminal, the nucleotides at positions 5, 7, 8, and 9 of the nucleotide sequence I in the sense strand of the siRNA are fluoro modified nucleotides, and the nucleotides at the other positions of the sense strand of the siRNA are methoxy modified nucleotides; and in the direction from 5' terminal to 3' terminal, the nucleotides at positions 2, 6, 8, 9, 14, and 16 of the nucleotide sequence II in the antisense strand of the siRNA are fluoro modified nucleotides, and the nucleotides at the other positions in the antisense strand of the siRNA are methoxy modified nucleotides;

35

40        Alternatively, in the direction from 5' terminal to 3' terminal, the nucleotides at positions 5, 7, 8, and 9 of the nucleotide sequence I in the sense strand of the siRNA are fluoro modified nucleotides, and the nucleotides at the other positions in the sense strand of the siRNA are methoxy modified nucleotides; and in the direction from 5' terminal to 3' terminal, the nucleotides at positions 2, 6, 14, and 16 of the nucleotide sequence II in the

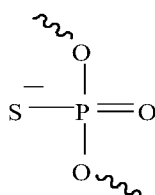
antisense strand of the siRNA are fluoro modified nucleotides, and the nucleotides at the other positions in the antisense strand of the siRNA are methoxy modified nucleotides.

Alternatively, in the direction from 5' terminal to 3' terminal, the nucleotides at positions 7, 8 and 9 of the nucleotide sequence I in the sense strand of the siRNA are fluoro modified nucleotides, and the nucleotides at the other positions in the sense strand of the siRNA are methoxy modified nucleotides; and in the direction from 5' terminal to 3' terminal, the nucleotides at positions 2, 6, 14, and 16 of the nucleotide sequence II in the antisense strand of the siRNA are fluoro modified nucleotides, and the nucleotides at the other positions in the antisense strand of the siRNA are methoxy modified nucleotides.

In some embodiments, the siRNA of the present disclosure is any one of siPCSKa1-M1, siPCSKa1-M2, siPCSKa1-M3, siPCSKa2-M1, siPCSKa2-M2, siPCSKa2-M3, siPCSKb1-M1, siPCSKb1-M2, siPCSKb1-M3, siPCSKb2-M1, siPCSKb2-M2, siPCSKb2-M3, siPCSKc1-M1, siPCSKc1-M2, siPCSKc1-M3, siPCSKc2-M1, siPCSKc2-M2, siPCSKc2-M3, siPCSKd1-M1, siPCSKd1-M2, siPCSKd1-M3, siPCSKd2-M1, siPCSKd2-M2, siPCSKd2-M3, siPCSKe1-M1, siPCSKe1-M2, siPCSKe1-M3, siPCSKe2-M1, siPCSKe2-M2, siPCSKe2-M3, siPCSKf1-M1, siPCSKf1-M2, siPCSKf1-M3, siPCSKf2-M1, siPCSKf2-M2 or siPCSKf2-M3 listed in Tables 1a-1f.

The siRNAs with the above modifications not only have lower costs, but also cause that the ribonucleases in the blood cannot easily cleave the nucleic acid, thereby increasing the stability of the nucleic acid and rendering the nucleic acid to have stronger resistance against nuclease hydrolysis. Meanwhile, the siRNAs with the above modifications also exhibit higher inhibitory activity against the target mRNA.

In some embodiments, at least a portion of the phosphate groups in the phosphate-ribose backbone of at least one single strand in the sense strand and the antisense strand of the siRNA of the present disclosure are phosphate groups with modified groups. In some embodiments, the phosphate group with modified group(s) is a phosphorothioate group formed by substituting at least one oxygen atom in a phosphodiester bond in the phosphate groups with a sulfur atom. In some embodiments, the phosphate group with modified group(s) is a phosphorothioate group having a structure as shown by Formula (1):



Formula (1).

This modification stabilizes the double-stranded structure of the siRNA, thereby maintaining high specificity and high affinity of base pairing.

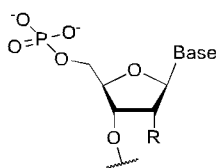
In some embodiments, in the siRNA of the present disclosure, the phosphorothioate linkage is located in at least one of the group consisting of the following positions: the position between the first and the second nucleotides at either terminal of the sense or antisense strand, the position between the second and the third nucleotides at either terminal of the sense or antisense strand, or any combination thereof. In some embodiments, the phosphorothioate linkage is located in all the above positions except 5' terminal of the sense strand. In some embodiments, the phosphorothioate linkage is located in all the above positions except 3' terminal of the sense strand. In some embodiments, the phosphorothioate linkage is located in at least one of the following positions:

- the position between the first and second nucleotides at 5' terminal of the sense strand;  
the position between the second and third nucleotides at 5' terminal of the sense strand;  
the position between the first and second nucleotides at 3' terminal of the sense strand;  
the position between the second and third nucleotides at 3' terminal of the sense strand;  
5 the position between the first and second nucleotides at 5' terminal of the antisense strand;  
the position between the second and third nucleotides at 5' terminal of the antisense strand;  
the position between the first and second nucleotides at 3' terminal of the antisense strand; and  
the position between the second and third nucleotides at 3' terminal of the antisense strand.
- 10 In some embodiments, the siRNA of the present disclosure is any one of siPCSKa1-M1S, siPCSKa1-M2S, siPCSKa1-M3S, siPCSKa2-M1S, siPCSKa2-M2S, siPCSKa2-M3S, siPCSKb1-M1S, siPCSKb1-M2S, siPCSKb1-M3S, siPCSKb2-M1S, siPCSKb2-M2S, siPCSKb2-M3S, siPCSKc1-M1S, siPCSKc1-M2S, siPCSKc1-M3S, siPCSKc2-M1S, siPCSKc2-M2S, siPCSKc2-M3S, siPCSKd1-M1S, siPCSKd1-M2S, siPCSKd1-M3S, siPCSKd2-M1S, siPCSKd2-M2S, siPCSKd2-M3S, siPCSKe1-M1S, siPCSKe1-M2S,  
15 siPCSKe1-M3S, siPCSKe2-M1S, siPCSKe2-M2S, siPCSKe2-M3S, siPCSKf1-M1S, siPCSKf1-M2S, siPCSKf1-M3S, siPCSKf2-M1S, siPCSKf2-M2S or siPCSKf2-M3S listed in Tables 1a-1f.

In some embodiments, the nucleotide at 5'-terminal in the antisense strand of the siRNA is a 5'-phosphate nucleotide or a 5'-phosphate analogue modified nucleotide.

20

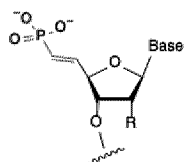
Typical 5'-phosphate nucleotides or 5'-phosphate analogue modified nucleotides are well known to those skilled in the art. For example, the 5'-phosphate nucleotides may have the following structure:



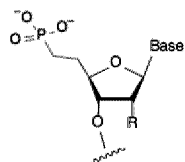
Formula (2).

25

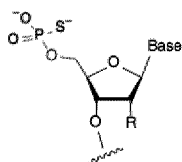
As another example, *Anastasia Khvorova and Jonathan K. Watts, The chemical evolution of oligonucleotide therapies of clinical utility. Nature Biotechnology, 2017, 35(3): 238-48* discloses the following four 5'-phosphate analogue modified nucleotides:



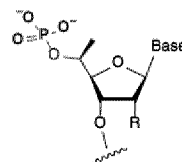
Formula (3)



Formula (4)



Formula (5)



Formula (6)

30

wherein R is selected from H, OH, methoxy, and F;  
“Base” represents a base selected from A, U, C, G, or T.

- 35 In some embodiments, the 5'-phosphate nucleotide is a nucleotide with 5'-phosphate as shown by Formula (2); the 5'-phosphate analogue modified nucleotide is a nucleotide with vinylphosphonate (5'-(E)-vinylphosphonate, E-VP) modification as shown by Formula (3), or a phosphorothioate modified nucleotide as shown by Formula (5).

In some embodiments, the siRNA of the present disclosure is any one of siPCSKa1-M1P1, siPCSKa1-M2P1, siPCSKa1-M3P1, siPCSKa2-M1P1, siPCSKa2-M2P1, siPCSKa2-M3P1, siPCSKb1-M1P1, siPCSKb1-M2P1, siPCSKb1-M3P1, siPCSKb2-M1P1, siPCSKb2-M2P1, siPCSKb2-M3P1, siPCSKc1-M1P1, siPCSKc1-M2P1, siPCSKc1-M3P1, siPCSKc2-M1P1, siPCSKc2-M2P1, siPCSKc2-M3P1, siPCSKd1-M1P1, siPCSKd1-M2P1, siPCSKd1-M3P1, siPCSKd2-M1P1, siPCSKd2-M2P1, siPCSKd2-M3P1, siPCSKe1-M1P1, siPCSKe1-M2P1, siPCSKe1-M3P1, siPCSKe2-M1P1, siPCSKe2-M2P1, siPCSKe2-M3P1, siPCSKf1-M1P1, siPCSKf1-M2P1, siPCSKf1-M3P1, siPCSKf2-M1P1, siPCSKf2-M2P1, siPCSKf2-M3P1, siPCSKa1-M1SP1, siPCSKa1-M2SP1, siPCSKa1-M3SP1, siPCSKa2-M1SP1, siPCSKa2-M2SP1, siPCSKa2-M3SP1, siPCSKb1-M1SP1, siPCSKb1-M2SP1, siPCSKb1-M3SP1, siPCSKb2-M1SP1, siPCSKb2-M2SP1, siPCSKb2-M3SP1, siPCSKc1-M1SP1, siPCSKc1-M2SP1, siPCSKc1-M3SP1, siPCSKc2-M1SP1, siPCSKc2-M2SP1, siPCSKc2-M3SP1, siPCSKd1-M1SP1, siPCSKd1-M2SP1, siPCSKd1-M3SP1, siPCSKd2-M1SP1, siPCSKd2-M2SP1, siPCSKd2-M3SP1, siPCSKe1-M1SP1, siPCSKe1-M2SP1, siPCSKe1-M3SP1, siPCSKe2-M1SP1, siPCSKe2-M2SP1, siPCSKe2-M3SP1, siPCSKf1-M1SP1, siPCSKf1-M2SP1, siPCSKf1-M3SP1, siPCSKf2-M1SP1, siPCSKf2-M2SP1 and siPCSKf2-M3SP1 listed in Tables 1a-1f.

### **Seventh siRNA**

According to the present disclosure, the siRNA may be a seventh siRNA.

20

The seventh siRNA comprises a sense strand and an antisense strand, and each nucleotide in the seventh siRNA is independently a modified or unmodified nucleotide; wherein the sense strand comprises a nucleotide sequence I, and the antisense strand comprises a nucleotide sequence II; the nucleotide sequence I and the nucleotide sequence II are at least partly reverse complementary to form a double-stranded region; wherein, the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 399 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 400 have an equal length and no more than 3 nucleotide differences:

30 5'-AGACCUGUUUUGCUUUUGZ<sub>25</sub>-3' (SEQ ID NO: 399);

5'-Z<sub>26</sub>CAAAAGCAAAACAGGUCU-3' (SEQ ID NO: 400),

wherein, Z<sub>25</sub> is U and Z<sub>26</sub> is A, and

the nucleotide sequence I comprises a nucleotide Z<sub>27</sub> at the position corresponding to Z<sub>25</sub>, the nucleotide sequence II comprises a nucleotide Z<sub>28</sub> at the position corresponding to Z<sub>26</sub>, wherein Z<sub>28</sub> is the first nucleotide at 5' terminal of the antisense strand.

35

In some embodiments, the sense strand comprises only the nucleotide sequence I, and the antisense strand comprises only the nucleotide sequence II.

In some embodiments, the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 399 have no more than 1 nucleotide difference, and/or the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 400 have no more than 1 nucleotide difference.

40

In some embodiments, the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 400 includes a difference at the position Z<sub>28</sub>, wherein Z<sub>28</sub> is selected from G, U or C. In some embodiments, the nucleotide difference is a difference at the position Z<sub>28</sub>, wherein Z<sub>28</sub> is selected from G, U or C. In some embodiments, Z<sub>27</sub> is a nucleotide complementary to Z<sub>28</sub>.

45

In some embodiments, the nucleotide difference between the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 399 includes a difference of the 9th nucleotide Z counted from 5' terminal of the nucleotide sequence I, wherein Z is dT.

5

In some embodiments, the nucleotide difference between the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 399 includes a difference at the position  $Z_{27}$ , wherein  $Z_{27}$  is selected from A, G or C.

10 In some embodiments, the nucleotide difference between the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 399 have 2 nucleotide differences, and the nucleotide sequence I and the nucleotide sequence II are completely reverse complementary to each other, wherein the 2 nucleotide differences are differences at the positions Z and  $Z_{27}$ , and Z is dT and  $Z_{27}$  is selected from A, G or C.

15 The siRNAs with the above-mentioned nucleotide differences have higher inhibitory ability against the target mRNA, and these siRNAs comprising the nucleotide differences are within the protection scope of the present disclosure.

In some embodiments, the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 401, and the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 402:

20 5'-AGACCUGUdTUUGCuuuUG $Z_{27}$ -3' (SEQ ID NO: 401);

5'- $Z_{28}$ CAAAAGCAAAACAGGUCU-3' (SEQ ID NO: 402),

Wherein dT is a thymine deoxynucleotide, the  $Z_{28}$  is the first nucleotide at 5' terminal of the antisense strand,  $Z_{27}$  is selected from A, U, G, or C, and  $Z_{28}$  is a nucleotide complementary to  $Z_{27}$ ; in some embodiments,  $Z_{27}$  is U, and  $Z_{28}$  is A.

25

In some embodiments, for the seventh siRNA, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 403, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 404:

5'-AGACCUGUdTUUGCuuuUG $Z_{27}$ -3' (SEQ ID NO: 403);

30 5'- $Z_{28}$ CAAAAGCAAAACAGGUCUAG-3' (SEQ ID NO: 404);

Alternatively, the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 405, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 406:

5'-CUAGACCUGUdTUUGCuuuUG $Z_{27}$ -3' (SEQ ID NO: 405);

5'- $Z_{28}$ CAAAAGCAAAACAGGUCUAGAA-3' (SEQ ID NO: 406);

35 In some embodiments, the siRNA of the present disclosure is siPCSKg3 or siPCSKg4 listed in Table 1g.

In some embodiments, in the above-mentioned siRNAs with nucleotide differences at positions Z and/or  $Z_{27}$ , at least some of the nucleotides are modified nucleotides, the modified nucleotides are fluoro modified nucleotides or non-fluoro modified nucleotides, the fluoro modified nucleotides are located in the nucleotide sequence I and the nucleotide sequence II, and in the direction from 5' terminal to 3' terminal, the nucleotides at least at positions 2, 6, 14, and 16 of the nucleotide sequence II are fluoro modified nucleotides.

40

In some embodiments, the siRNA of the present disclosure is any one of siPCSKg3-M4, siPCSKg4-M5, siPCSKg3-M4S, siPCSKg4-M5S, siPCSKg3-M4P1, siPCSKg4-M5P1, siPCSKg3-M4SP1 or siPCSKg4-M5SP1 listed in Table 1g.

45

The inventors of the present disclosure surprisingly found that the siRNAs of the present disclosure have significantly enhanced plasma and lysosomal stability, while exhibiting higher inhibitory activity against the target mRNA.

- 5 The siRNAs of the present disclosure can be obtained by conventional methods for preparing siRNAs in the art, e.g., solid phase synthesis method and liquid phase synthesis method. Commercial customization services have already been available for solid phase synthesis. A modified nucleotide group can be introduced into the siRNA of the present disclosure by using a nucleotide monomer having the corresponding modification. The method for preparing a nucleotide monomer having the corresponding modification and the method for  
10 introducing a modified nucleotide group into an siRNA are also well known to those skilled in the art.

### **Pharmaceutical Composition**

The present disclosure provides a pharmaceutical composition, comprising the above siRNA as an active ingredient and a pharmaceutically acceptable carrier.

- 15 The pharmaceutically acceptable carrier may be a conventional carrier in the field of siRNA administration, for example, but not limited to, one or more of magnetic nanoparticles (such as Fe<sub>3</sub>O<sub>4</sub> and Fe<sub>2</sub>O<sub>3</sub>-based nanoparticle), carbon nanotubes, mesoporous silicon, calcium phosphate nanoparticles, polyethylenimine (PEI), polyamidoamine (PAMAM) dendrimer, poly(L-lysine) (PLL), chitosan, 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), poly(D&L-lactic/glycolic acid) copolymer (PLGA), poly(2-aminoethyl ethylene phosphate) (PPEEA), poly(2-dimethylaminoethyl methacrylate) (PDMAEMA), and derivatives thereof.  
20

- In the pharmaceutical composition, the contents of the siRNA and the pharmaceutically acceptable carrier are not specially required, and can be conventional contents of individual components. In some embodiments, the weight ratio of the siRNA to the pharmaceutically acceptable carrier is 1: (1-500), and in some embodiments,  
25 the weight ratio is 1: (1-50).

- In some embodiments, the pharmaceutical composition may also contain other pharmaceutically acceptable excipients, which may be one or more of various conventional formulations or compounds in the art. For example, said other pharmaceutically acceptable excipients may comprise at least one of a pH buffer, a  
30 protection agent and an osmotic pressure regulator.

- The pH buffer may be a tris(hydroxymethyl) aminomethane hydrochloride buffer solution with a pH of 7.5-8.5, and/or a phosphate buffer solution with a pH of 5.5-8.5, such as a phosphate buffer solution with a pH of  
35 5.5-8.5.

- The protection agent may be at least one of inositol, sorbitol, sucrose, trehalose, mannose, maltose, lactose, and glucose. The content of the protection agent may be from 0.01 wt % to 30 wt % based on the total weight of the pharmaceutical composition.  
40

- The osmotic pressure regulator may be sodium chloride and/or potassium chloride. The content of the osmotic pressure regulator renders the osmotic pressure of the pharmaceutical composition to be 200-700 milliosmol/kg. Depending on the desired osmotic pressure, those skilled in the art can readily determine the content of the osmotic pressure regulator.  
45

15

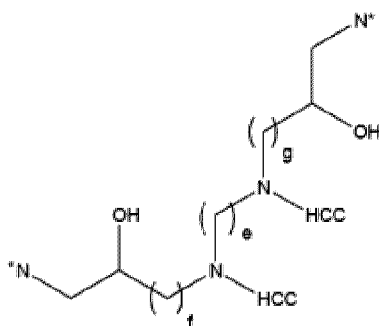
$$\left[ \begin{array}{c} \text{R}_{104} \quad \text{R}_{105} \\ \diagdown \quad \diagup \\ \text{C} \text{---} \text{Y}_{101} \text{---} \text{X}_{101} \text{---} \text{R}_{101} \\ \diagup \quad \diagdown \\ \text{N} \text{---} \text{R}_{103} \\ \diagup \quad \diagdown \\ \text{C} \text{---} \text{Z}_{101} \text{---} \text{X}_{102} \text{---} \text{R}_{102} \\ \diagdown \quad \diagup \\ \text{R}_{106} \quad \text{R}_{107} \end{array} \right]_n \text{---} \left[ \text{Z}_{101} \text{---} \text{X}_{102} \text{---} \text{R}_{102} \right]_p$$

20

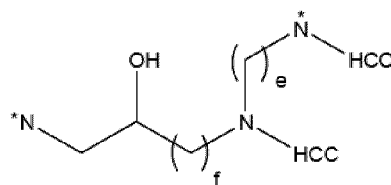
each  $Y_{101}$  or  $Z_{101}$  independently of one another is selected from C=O, C=S, S=O, CH-OH or SO<sub>2</sub>;

30

if at least one of n and m is 2, then R<sub>103</sub> and nitrogen in Formula (201) form a structure as shown by Formula (202) or (203):



Formula (202)

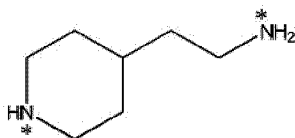


Formula (203);

wherein g, e or f independently of one another is an integer of 1-6; "HCC" represents a hydrocarbon chain, and each \*N represents a nitrogen atom shown in Formula (201).

In some embodiments,  $R_{103}$  is a polyamine. In other embodiments,  $R_{103}$  is a ketal. In some embodiments,  $R_{101}$  and  $R_{102}$  in the Formula (201) independently of one another are any substituted or unsubstituted, branched or linear alkyl or alkenyl, wherein the alkyl or alkenyl has 3-20 carbon atoms (such as 8-18 carbon atoms) and 0-4 double bonds (such as 0-2 double bonds).

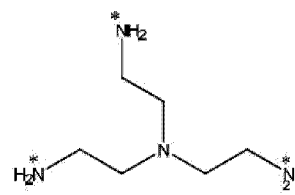
In some embodiments, if n and m independently of one another are 1 or 3,  $R_{103}$  is any of the following Formulae (204)-(213):



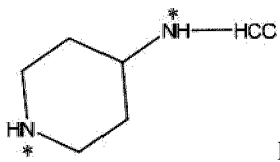
Formula (204),



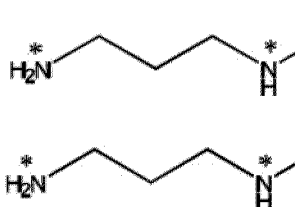
Formula (205),



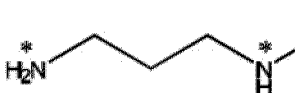
Formula (206),



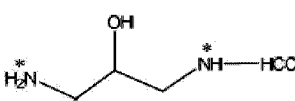
Formula (207),



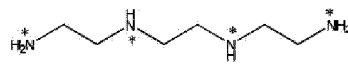
Formula (208),



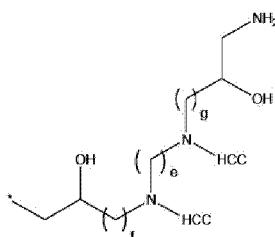
Formula (209),



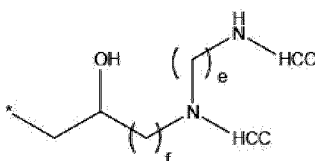
Formula (210),



Formula (211),



Formula (212) and

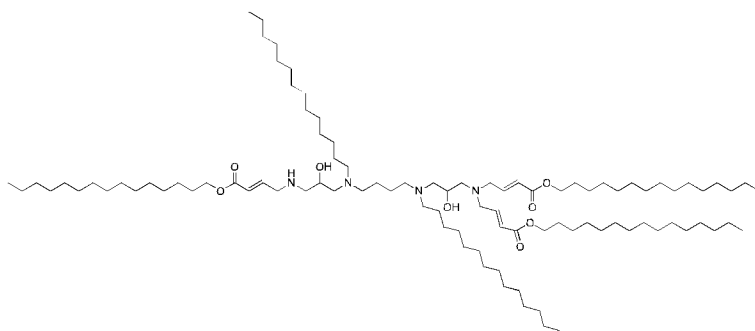


Formula (213);

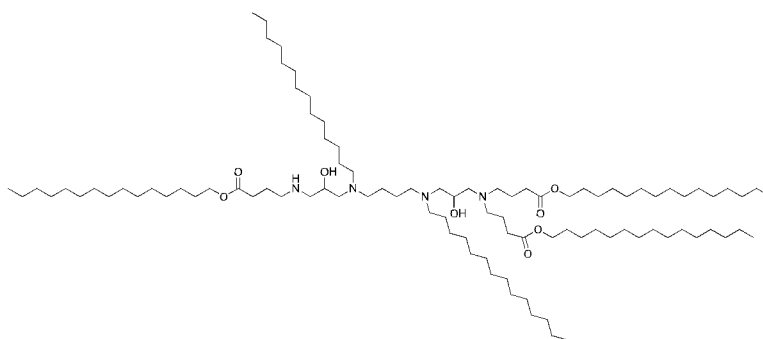
wherein, in Formulae (204)-(213), g, e and f independently of one another are an integer of 1-6, each "HCC" represents a hydrocarbon chain, and each \* represents a potential attachment point of R<sub>103</sub> to the nitrogen atom in Formula (201), wherein each H at any \* position can be replaced to achieve the attachment to the nitrogen atom in Formula (201).

The compound as shown by Formula (201) may be prepared as described in CN103380113A.

In some embodiments, the organic amine is an organic amine as shown by Formula (214) and/or an organic amine as shown by Formula (215):



Formula (214),



Formula (215);

The helper lipid is cholesterol, cholesterol analogs and/or cholesterol derivatives.

The PEGylated lipid is 1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine-N-[methoxy(polyethylene glycol)]-2000.

In some embodiments, the molar ratio among the organic amine, the helper lipid, and the PEGylated lipid in the pharmaceutical composition is (19.7-80): (19.7-80): (0.3-50), for example, the molar ratio may be (50-70): (20-40): (3-20).

In some embodiments, the pharmaceutical composition particles formed by the siRNA of the present disclosure and the above amine-containing transfection reagents have an average diameter from about 30 nm to about 200 nm, typically from about 40 nm to about 135 nm, and more typically, the average diameter of the liposome particles is from about 50 nm to about 120 nm, from about 50 nm to about 100 nm, from about 60 nm to about 90 nm, or from about 70 nm to about 90 nm; for example, the average diameter of the liposome particles is about 30, 40, 50, 60, 70, 75, 80, 85, 90, 100, 110, 120, 130, 140, 150 or 160 nm.

In some embodiments, in the pharmaceutical composition formed by the siRNA of the present disclosure and the above amine-containing transfection reagents, the weight ratio (weight/weight ratio) of the siRNA to total lipids, e.g., the organic amines, the helper lipids and/or the PEGylated lipids, ranges from about 1:1 to about 1:50, from about 1:1 to about 1:30, from about 1:3 to about 1:20, from about 1:4 to about 1:18, from about 1:5 to about 1:17, from about 1:5 to about 1:15, from about 1:5 to about 1:12, from about 1:6 to about 1:12, or from about 1:6 to about 1:10. For example, the weight ratio of the siRNA of the present disclosure to total lipids is about 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, or 1:18.

In some embodiments, the pharmaceutical composition may be marketed with each component being separate, and used in the form of a liquid formulation. In some embodiments, the pharmaceutical composition formed by the siRNA of the present disclosure and the above pharmaceutically acceptable carrier may be prepared by various known processes, except for replacing the existing siRNA with the siRNA of the present disclosure. In some specific embodiments, the pharmaceutical composition may be prepared according to the following process:

The organic amines, helper lipids and PEGylated lipids are suspended in alcohol at a molar ratio as described above and mixed homogeneously to yield a lipid solution; the alcohol is used in an amount such that the resultant lipid solution is present at a total mass concentration of 2 to 25 mg/mL (e.g., 8 to 18 mg/mL). The alcohol is a pharmaceutically acceptable alcohol, such as an alcohol that is in liquid form at about room temperature, for example, one or more of ethanol, propylene glycol, benzyl alcohol, glycerol, PEG 200, PEG 300, and PEG 400, such as ethanol.

The siRNA of the present disclosure is dissolved in a buffered salt solution to produce an aqueous solution of the siRNA. The buffered salt solution has a concentration of 0.05 to 0.5 M, such as 0.1 to 0.2 M. The pH of the buffered salt solution is adjusted to 4.0 to 5.5, such as 5.0 to 5.2. The buffered salt solution is used in an amount such that the siRNA is present at a concentration of less than 0.6 mg/ml, such as 0.2 to 0.4 mg/mL. The buffered salt may be one or more selected from the group consisting of soluble acetate and soluble citrate, such as sodium acetate and/or potassium acetate.

The lipid solution and the aqueous solution of the siRNA are mixed. The product obtained by mixing is incubated at a temperature of 40 to 60°C for at least 2 minutes (e.g., 5 to 30 minutes) to produce an incubated lipid formulation. The volume ratio of the lipid solution to the aqueous solution of the siRNA is 1: (2-5).

The incubated lipid formulation is concentrated or diluted, purified to remove impurities, and then sterilized to afford the pharmaceutical composition of the present disclosure, which has the following physicochemical parameters: a pH of 6.5 to 8, an encapsulation percentage of more than 80%, a particle size of 40 to 200 nm, a polydispersity index of less than 0.30, and an osmotic pressure of 250 to 400 mOsm/kg. For example, the physicochemical parameters may be as follows: a pH of 7.2 to 7.6, an encapsulation percentage of more than 90%, a particle size of 60 to 100 nm, a polydispersity index of less than 0.20, and an osmotic pressure of 300 to 400 mOsm/kg.

Therein, the concentration or dilution step may be performed before, after or simultaneously with removal of the impurities. The method for removing impurities may be any of various existing methods, for example, ultrafiltration with 100 kDa hollow fiber column and PBS at pH 7.4 as ultrafiltration exchange solution using tangential flow system. The method for sterilization may be any of various existing methods, such as filtration sterilization on a 0.22 µm filter.

**siRNA conjugate**

The present disclosure provides an siRNA conjugate comprising the above siRNA and a conjugation group conjugatively linked to the siRNA.

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Generally speaking, the conjugation group comprises at least one pharmaceutically acceptable targeting group and an optional linker. Moreover, the siRNA, the linker and the targeting group are sequentially linked. In some embodiments, the number of the targeting groups is 1 to 6. In some embodiments, the number of targeting groups is 2 to 4. The siRNA molecule may be non-covalently or covalently conjugated to the conjugation group, for example the siRNA molecule may be covalently conjugated to the conjugation group. The conjugation site between the siRNA and the conjugation group can be at 3' terminal or 5' terminal of the sense strand of the siRNA, or at 5' terminal of the antisense strand of the siRNA, and can be within the internal sequence of the siRNA. In some embodiments, the conjugation site between the siRNA and the conjugation group is at 3' terminal of the sense strand of the siRNA.

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In some embodiments, the conjugation group may be linked to the phosphate group, the 2'-hydroxy or the base of a nucleotide. The conjugation group may also be linked to the 3'-hydroxy group when the nucleotides are linked via a 2'-5'-phosphodiester bond. When the conjugation group is linked to a terminal of the siRNA, the conjugation group is typically linked to the phosphate group of a nucleotide; when the conjugation group is linked to an internal sequence of the siRNA, the conjugation group is typically linked to a ribose ring or a base. For various linking modes, reference may be made to: Muthiah Manoharan *et.al.* siRNA conjugates carrying sequentially assembled trivalent N-acetylgalactosamine linked through nucleosides elicit robust gene silencing in vivo in hepatocytes. ACS Chemical biology, 2015, 10(5): 1181-7.

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In some embodiments, the siRNA and the conjugation group can be linked by an acid-labile or reducible chemical bond, and these chemical bonds can be degraded under the acidic environment of cell endosomes, thereby making the siRNA be in free state. For non-degradable conjugation modes, the conjugation group can be linked to the sense strand of the siRNA, thereby minimizing the effect of conjugation on the activity of the siRNA.

30

In some embodiments, the pharmaceutically acceptable targeting group may be a conventional ligand in the field of siRNA administration, for example, various ligands as described in WO2009082607A2, which is incorporated herein by reference in its entirety.

35

In some embodiments, the pharmaceutically acceptable targeting group may be one or more selected from the ligands formed by the following targeting molecules or derivatives thereof: lipophilic molecules, such as cholesterol, bile acids, vitamins (such as vitamin E), lipid molecules with different chain lengths; polymers, such as polyethylene glycol; polypeptides, such as cell-penetrating peptide; aptamers; antibodies; quantum dots; saccharides, such as lactose, polylactose, mannose, galactose, N-acetylgalactosamine (GalNAc); folate; or receptor ligands expressed in hepatic parenchymal cells, such as asialoglycoprotein, asialo-sugar residue, lipoproteins (such as high density lipoprotein, low density lipoprotein and the like), glucagon, neurotransmitters (such as adrenaline), growth factors, transferrin and the like.

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In some embodiments, each ligand is independently selected from a ligand capable of binding to a cell surface receptor. In some embodiments, at least one ligand is a ligand capable of binding to a surface receptor of a hepatocyte. In some embodiments, at least one ligand is a ligand capable of binding to a surface receptor of a

mammalian hepatocyte. In some embodiments, at least one ligand is a ligand capable of binding to a surface receptor of a human hepatocyte. In some embodiments, at least one ligand is a ligand capable of binding to an asialoglycoprotein receptor (ASGPR) on the surface of hepatocytes. The types of these ligands are well-known to those skilled in the art and they typically serve the function of binding to specific receptor on the surface of the target cell, thereby mediating delivery of the siRNA linked to the ligand into the target cell.

In some embodiments, the pharmaceutically acceptable targeting group may be any ligand that binds to the asialoglycoprotein receptors (ASGPR) on the surface of mammalian hepatocytes. In some embodiments, each ligand is independently an asialoglycoprotein, such as asialoorosomucoid (ASOR) or asialofetuin (ASF). In some embodiments, the ligand is a saccharide or its derivatives.

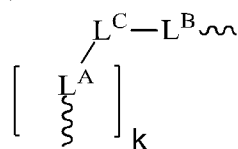
In some embodiments, at least one ligand is a saccharide. In some embodiments, each ligand is a saccharide. In some embodiments, at least one ligand is a monosaccharide, polysaccharide, modified monosaccharide, modified polysaccharide, or saccharide derivative. In some embodiments, at least one ligand may be a monosaccharide, disaccharide or trisaccharide. In some embodiments, at least one ligand is a modified saccharide. In some embodiments, each ligand is a modified saccharide. In some embodiments, each ligand is independently selected from a polysaccharide, modified polysaccharide, monosaccharide, modified monosaccharide, polysaccharide derivative, and monosaccharide derivative. In some embodiments, each ligand or at least one ligand may be independently selected from the group consisting of glucose and its derivatives, mannose and its derivatives, galactose and its derivatives, xylose and its derivatives, ribose and its derivatives, fucose and its derivatives, lactose and its derivatives, maltose and its derivatives, arabinose and its derivatives, fructose and its derivatives, and sialic acid.

In some embodiments, each ligand may be independently selected from the group consisting of D-mannopyranose, L-mannopyranose, D-arabinose, D-xylofuranose, L-xylofuranose, D-glucose, L-glucose, D-galactose, L-galactose,  $\alpha$ -D-mannofuranose,  $\beta$ -D-mannofuranose,  $\alpha$ -D-mannopyranose,  $\beta$ -D-mannopyranose,  $\alpha$ -D-glucopyranose,  $\beta$ -D-glucopyranose,  $\alpha$ -D-glucofuranose,  $\beta$ -D-glucofuranose,  $\alpha$ -D-fructofuranose,  $\alpha$ -D-fructopyranose,  $\alpha$ -D-galactopyranose,  $\beta$ -D-galactopyranose,  $\alpha$ -D-galactofuranose,  $\beta$ -D-galactofuranose, glucosamine, sialic acid, galactosamine, N-acetylgalactosamine, N-trifluoroacetylgalactosamine, N-propionylgalactosamine, N-n-butyrylgalactosamine, N-isobutyrylgalactosamine, 2-amino-3-O-[(R)-1-carboxyethyl]-2-deoxy- $\beta$ -D-glucopyranose, 2-deoxy-2-methylamino-L-glucopyranose, 4,6-dideoxy-4-formamido-2,3-di-O-methyl-D-mannopyranose, 2-deoxy-2-sulfoamino-D-glucopyranose, N-glycolyl- $\alpha$ -neuraminic acid, 5-thio- $\beta$ -D-glucopyranose, methyl 2,3,4-tris-O-acetyl-1-thio-6-O-trityl- $\alpha$ -D-glucopyranoside, 4-thio- $\beta$ -D-galactopyranose, ethyl 3,4,6,7-tetra-O-acetyl-2-deoxy-1,5-dithio- $\alpha$ -D-glucuheptopyranoside, 2,5-anhydro-D-allononitrile, ribose, D-ribose, D-4-thioribose, L-ribose, L-4-thioribose. Other options of the ligand may be found, for example, in the disclosure of CN105378082A, which is incorporated herein by reference in its entirety.

In some embodiments, the pharmaceutically acceptable targeting group in the siRNA conjugate may be galactose or N-acetylgalactosamine, wherein the galactose or N-acetylgalactosamine molecules may be mono-, bi-, tri-, or tetra-valent. It should be understood that the terms mono-, bi-, tri-, or tetra-valent described herein respectively mean that the molar ratio of the siRNA molecule to the galactose or N-acetylgalactosamine molecule in the siRNA conjugate is 1:1, 1:2, 1:3 or 1:4, wherein the siRNA conjugate is formed from the siRNA molecule and the conjugation group containing galactose or N-acetylgalactosamine molecule as the targeting group. In some embodiments, the pharmaceutically acceptable targeting group is N-acetylgalactosamine. In some embodiments, when the double-stranded oligonucleotide of the present disclosure is conjugated to a

conjugation group comprising N-acetylgalactosamine, the N-acetylgalactosamine molecule is trivalent or tetravalent. In some embodiments, when the siRNA of the present disclosure is conjugated to a conjugation group containing N-acetylgalactosamine, the N-acetylgalactosamine molecule is trivalent.

- 5 The targeting group can be linked to the siRNA molecule via an appropriate linker, and the appropriate linker can be selected by those skilled in the art according to the specific type of the targeting group. The types of these linkers and targeting groups and the linking modes with the siRNA may be found in the disclosure of WO2015006740A2, which is incorporated herein by reference in its entirety.
- 10 In some embodiments, when the targeting group is N-acetylgalactosamine, a suitable linker may have the following structure as shown by Formula (301):

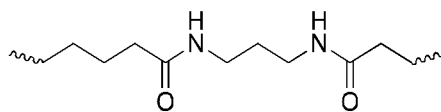


Formula (301)

15 wherein,

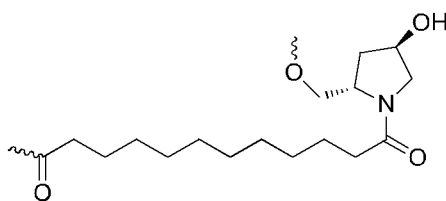
k is an integer of 1-3;

$L^A$  is an amide bond-comprising chain moiety that has a structure as shown by Formula (302), and two terminals of each  $L^A$  are respectively linked to the targeting group and the  $L^C$  moiety through an ether bond:



Formula (302);

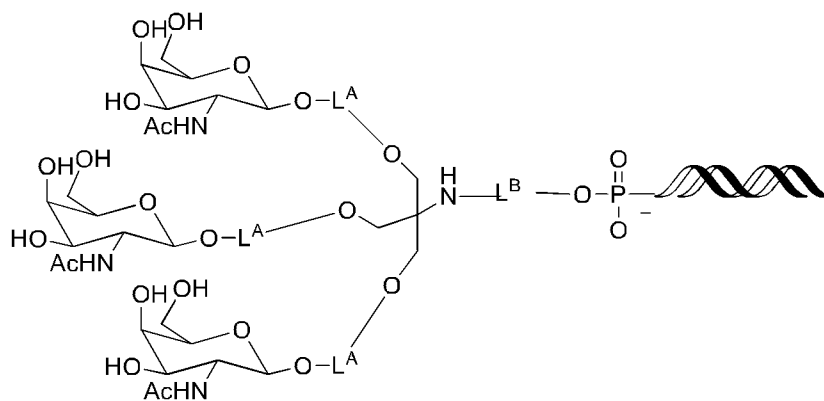
$L^B$  is a N-acylpyrrolidine-comprising chain moiety that has a structure as shown by Formula (303), wherein the chain moiety has a carbonyl group at one terminal and is linked to the  $L^C$  moiety through an amide bond, and has an oxy group at the other terminal and is linked to the siRNA via a phosphoester bond:



Formula (303);

- $L^C$  is a bivalent to tetravalent linking group based on hydroxymethyl aminomethane, dihydroxymethyl aminomethane or trihydroxymethyl aminomethane, and  $L^C$  is linked to  $L^A$  moieties through an ether bond via an oxygen atom, and is linked to  $L^B$  moiety through an amide bond via a nitrogen atom.

In some embodiments, when  $n=3$  and  $L^C$  is a tetravalent linking group based on trihydroxymethyl aminomethane, the siRNA conjugate formed by linking N-acetylgalactosamine molecules with an siRNA molecule via  $-(L^A)_3$ -trihydroxymethyl aminomethane- $L^B$ - as a linker has a structure as shown by Formula (304):

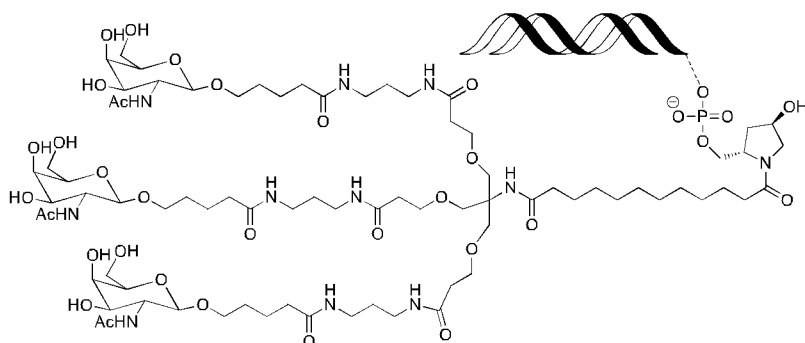


Formula (304),

wherein the double helix structure represents an siRNA.

- 5 Likewise, the conjugation site between the siRNA and the conjugation group can be at 3'-terminal or 5'-terminal of the sense strand of the siRNA, or at 5'-terminal of the antisense strand, or within the internal sequence of the siRNA.

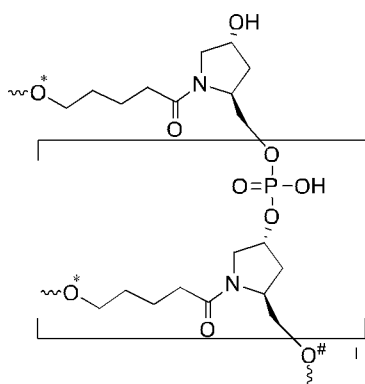
- 10 In some embodiments, the 3'-terminal of the sense strand of the siRNA of the present disclosure is covalently conjugated to three N-acetylgalactosamine (GalNAc) molecules via a linker  $-(L^A)_3$ -trihydroxymethyl aminomethane- $L^B$ -, to afford an siRNA conjugate in which the molar ratio of the siRNA molecule to the GalNAc molecule is 1:3 (hereinafter also referred to as (GalNAc)<sub>3</sub>-siRNA), and this conjugate has a structure as shown by Formula (305):



15 Formula (305),

wherein the double helix structure represents an siRNA; and the linker is linked to 3'-terminal of the sense strand of the siRNA.

- 20 In some embodiments, when the targeting group is N-acetylgalactosamine, a suitable linker may have a structure as shown by Formula (306):



Formula (306)

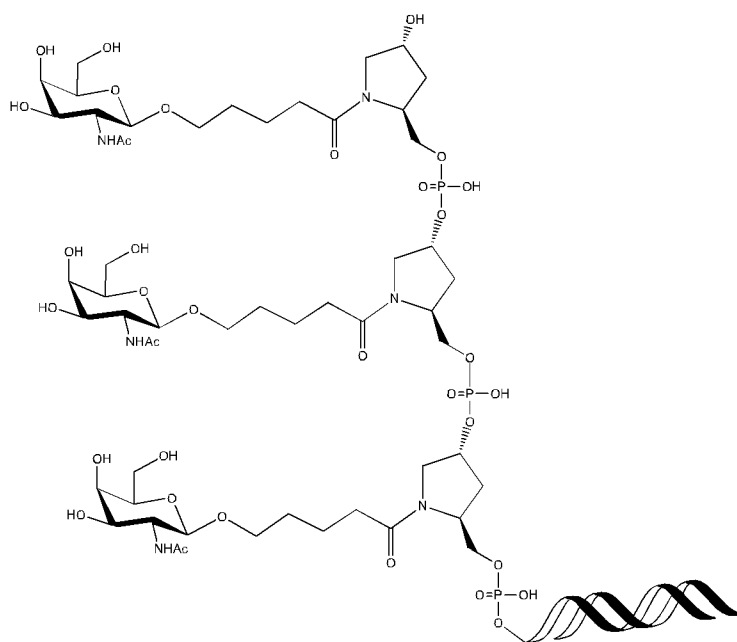
wherein,

5 l is an integer of 0-3;

\* represents a site on the linker linked to the targeting group via an ether bond; and

# represents a site on the linker linked to the siRNA via a phosphoester bond.

In some specific embodiments, when  $l=2$ , the siRNA conjugate has a structure as shown by Formula (307):

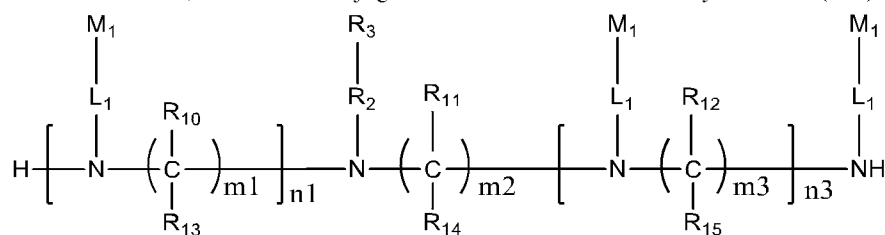


Formula (307),

wherein, the double helix structure denotes an siRNA; and the linker is linked to 3'-terminal of the sense strand of the siRNA.

The above conjugates can be synthesized according to the method described in detail in the prior art. For example, WO2015006740 A2 describes in detail the preparation methods of various conjugates. The siRNA conjugate of the present disclosure may be obtained by the methods well-known to those skilled in the art. For example, WO2014025805A1 describes the preparation method of the conjugate having the structure as shown by Formula (305). Rajeev *et al.*, ChemBioChem 2015, 16, 903-908 describes the preparation method of the conjugate having the structure as shown by Formula (307).

In some embodiments, the siRNA conjugate has a structure as shown by Formula (308):



Formula (308),

5

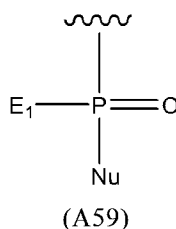
wherein,

$n1$  is an integer of 1-3, and  $n3$  is an integer of 0-4;

each  $m1$ ,  $m2$ , or  $m3$  independently of one another is an integer of 2-10;

10  $R_{10}$ ,  $R_{11}$ ,  $R_{12}$ ,  $R_{13}$ ,  $R_{14}$ , or  $R_{15}$  independently of one another is H, or selected from the group consisting of  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  haloalkyl, and  $C_1$ - $C_{10}$  alkoxy,

$R_3$  is a group having a structure as shown by Formula (A59):



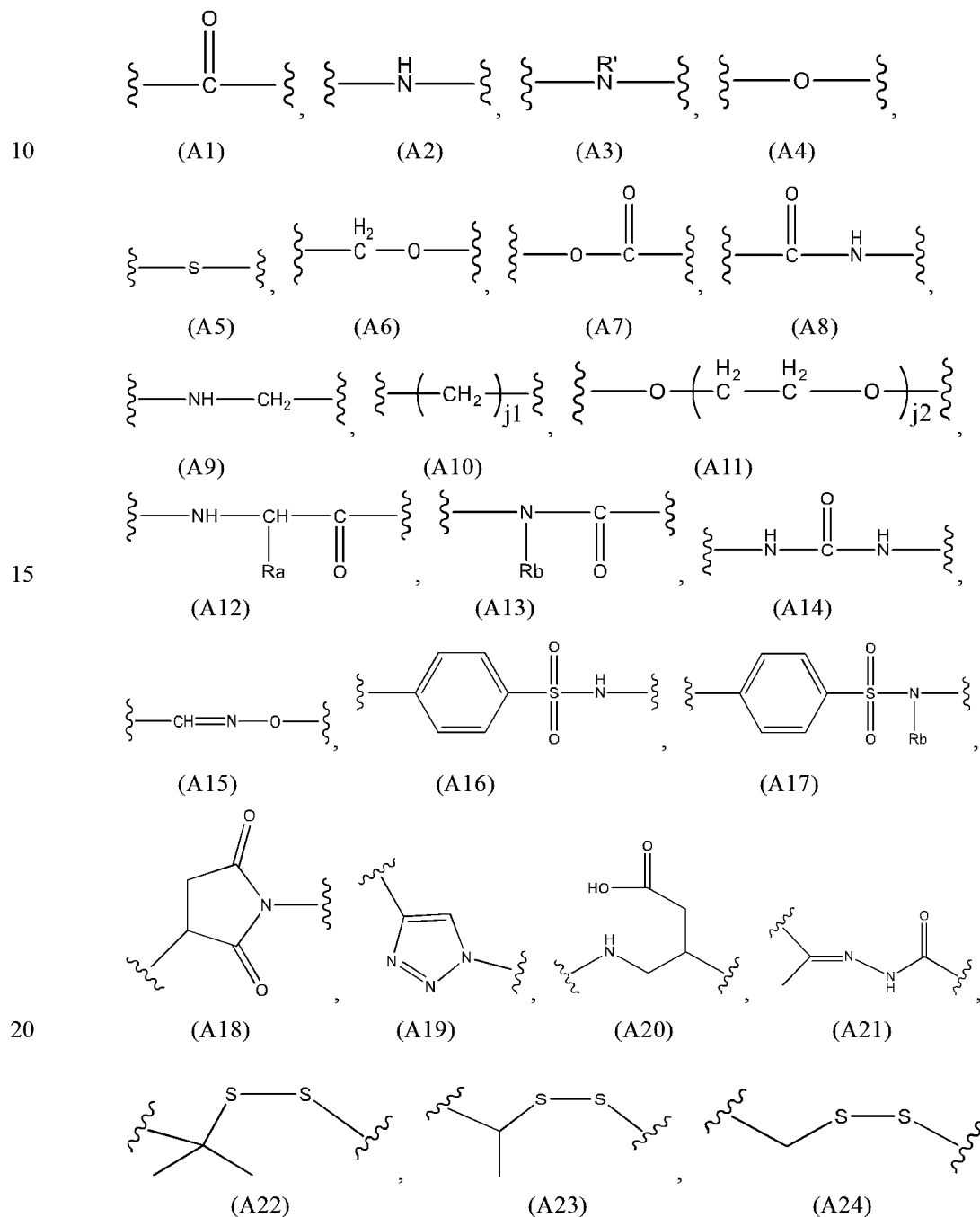
15 wherein  $E_1$  is OH, SH or  $BH_2$ ; and Nu is the siRNA of the present disclosure;

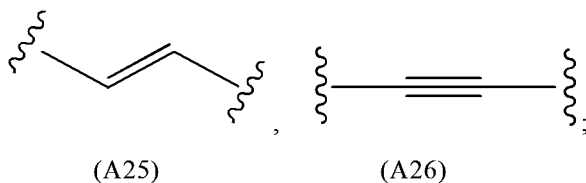
$R_2$  is a linear alkylene of 1 to 20 carbon atoms in length, wherein one or more carbon atoms are optionally replaced with any one or more groups selected from the group consisting of:  $C(O)$ , NH, O, S,  $CH=N$ ,  $S(O)_2$ ,  $C_2$ - $C_{10}$  alkenylene,  $C_2$ - $C_{10}$  alkynylene,  $C_6$ - $C_{10}$  arylene,  $C_3$ - $C_{18}$  heterocyclylene, and  $C_5$ - $C_{10}$  heteroarylene, and wherein  $R_2$  optionally has any one or more substituents selected from the group consisting of:  $C_1$ - $C_{10}$  alkyl,  $C_6$ - $C_{10}$  aryl,  $C_5$ - $C_{10}$  heteroaryl,  $C_1$ - $C_{10}$  haloalkyl,  $-OC_1$ - $C_{10}$  alkyl,  $-OC_1$ - $C_{10}$  alkylphenyl,  $-C_1$ - $C_{10}$  alkyl-OH,  $-OC_1$ - $C_{10}$  haloalkyl,  $-SC_1$ - $C_{10}$  alkyl,  $-SC_1$ - $C_{10}$  alkylphenyl,  $-C_1$ - $C_{10}$  alkyl-SH,  $-SC_1$ - $C_{10}$  haloalkyl, halo, -OH, -SH, -NH<sub>2</sub>,  $-C_1$ - $C_{10}$  alkyl-NH<sub>2</sub>,  $-N(C_1$ - $C_{10}$  alkyl)( $C_1$ - $C_{10}$  alkyl),  $-NH(C_1$ - $C_{10}$  alkyl),  $N(C_1$ - $C_{10}$  alkyl)( $C_1$ - $C_{10}$  alkylphenyl),  $NH(C_1$ - $C_{10}$  alkylphenyl), cyano, nitro,  $-CO_2H$ ,  $-C(O)OC_1$ - $C_{10}$  alkyl,  $-CON(C_1$ - $C_{10}$  alkyl)( $C_1$ - $C_{10}$  alkyl),  $-CONH(C_1$ - $C_{10}$  alkyl),  $-CONH_2$ ,  $-NHC(O)(C_1$ - $C_{10}$  alkyl),  $-NHC(O)(phenyl)$ ,  $-N(C_1$ - $C_{10}$  alkyl) $C(O)(C_1$ - $C_{10}$  alkyl),  $-N(C_1$ - $C_{10}$  alkyl) $C(O)(phenyl)$ ,  $-C(O)C_1$ - $C_{10}$  alkyl,  $-C(O)C_1$ - $C_{10}$  alkylphenyl,  $-C(O)C_1$ - $C_{10}$  haloalkyl,  $-OC(O)C_1$ - $C_{10}$  alkyl,  $-SO_2(C_1$ - $C_{10}$  alkyl),  $-SO_2(phenyl)$ ,  $-SO_2(C_1$ - $C_{10}$  haloalkyl),  $-SO_2NH_2$ ,  $-SO_2NH(C_1$ - $C_{10}$  alkyl),  $-SO_2NH(phenyl)$ ,  $-NHSO_2(C_1$ - $C_{10}$  alkyl),  $-NHSO_2(phenyl)$ , and  $-NHSO_2(C_1$ - $C_{10}$  haloalkyl);

30 each  $L_1$  is independently a linear alkylene of 1 to 70 carbon atoms in length, wherein one or more carbon atoms are optionally replaced with any one or more groups selected from the group consisting of:  $C(O)$ , NH, O, S,  $CH=N$ ,  $S(O)_2$ ,  $C_2$ - $C_{10}$  alkenylene,  $C_2$ - $C_{10}$  alkynylene,  $C_6$ - $C_{10}$  arylene,  $C_3$ - $C_{18}$  heterocyclylene, and  $C_5$ - $C_{10}$  heteroarylene, and wherein  $L_1$  optionally has any one or more substituents selected from the group consisting of:  $C_1$ - $C_{10}$  alkyl,  $C_6$ - $C_{10}$  aryl,  $C_5$ - $C_{10}$  heteroaryl,  $C_1$ - $C_{10}$  haloalkyl,  $-OC_1$ - $C_{10}$  alkyl,  $-OC_1$ - $C_{10}$  alkylphenyl,  $-C_1$ - $C_{10}$  alkyl-OH,  $-OC_1$ - $C_{10}$  haloalkyl,  $-SC_1$ - $C_{10}$  alkyl,  $-SC_1$ - $C_{10}$  alkylphenyl,  $-C_1$ - $C_{10}$  alkyl-SH,  $-SC_1$ - $C_{10}$  haloalkyl, halo, -OH, -SH, -NH<sub>2</sub>,  $-C_1$ - $C_{10}$  alkyl-NH<sub>2</sub>,  $-N(C_1$ - $C_{10}$  alkyl)( $C_1$ - $C_{10}$  alkyl),  $-NH(C_1$ - $C_{10}$  alkyl),  $N(C_1$ - $C_{10}$  alkyl)( $C_1$ - $C_{10}$  alkylphenyl),  $NH(C_1$ - $C_{10}$  alkylphenyl), cyano, nitro,  $-CO_2H$ ,

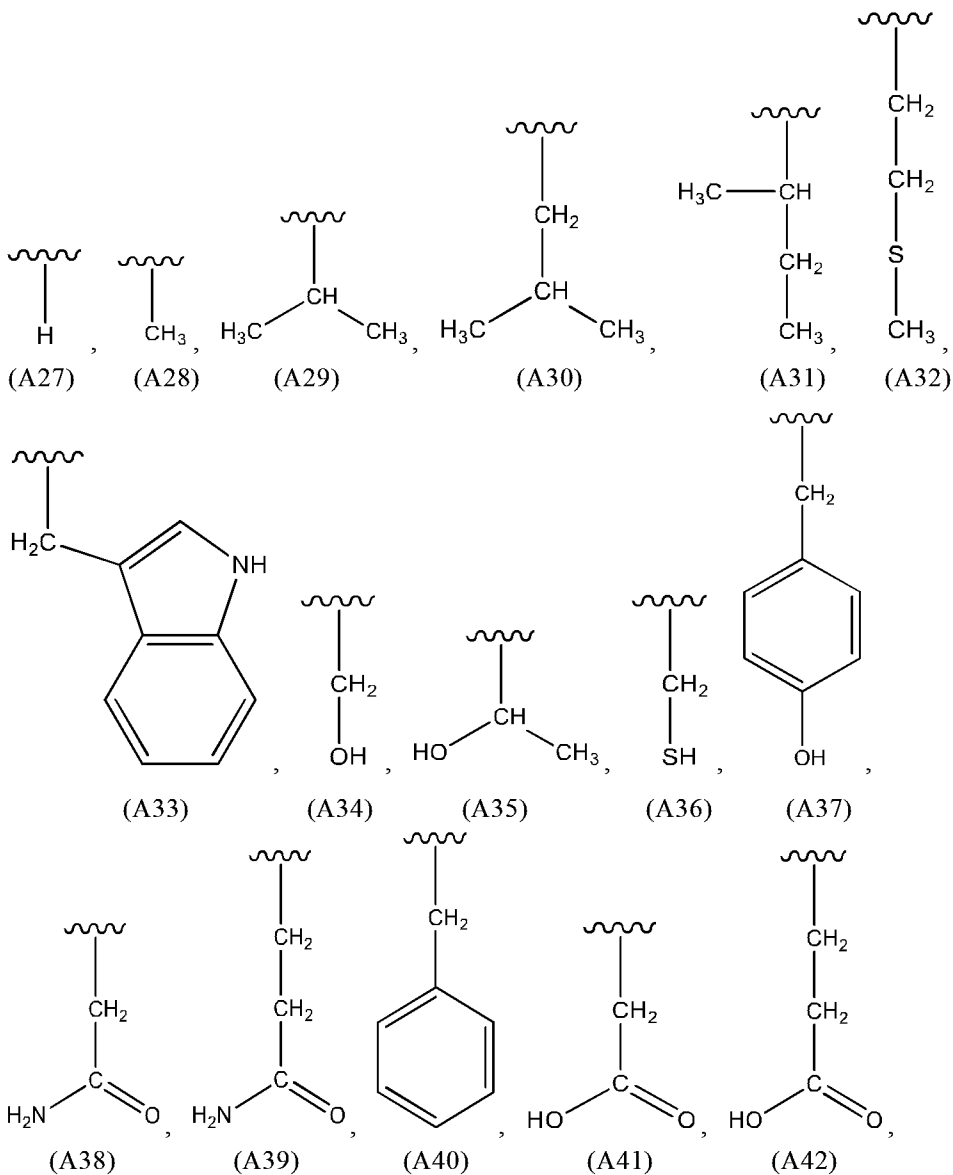
- 5 -C(O)OC<sub>1</sub>-C<sub>10</sub> alkyl, -CON(C<sub>1</sub>-C<sub>10</sub> alkyl)(C<sub>1</sub>-C<sub>10</sub> alkyl), -CONH(C<sub>1</sub>-C<sub>10</sub> alkyl), -CONH<sub>2</sub>, -NHC(O)(C<sub>1</sub>-C<sub>10</sub> alkyl), -NHC(O)(phenyl), -N(C<sub>1</sub>-C<sub>10</sub> alkyl)C(O)(C<sub>1</sub>-C<sub>10</sub> alkyl), -N(C<sub>1</sub>-C<sub>10</sub> alkyl)C(O)(phenyl), -C(O)C<sub>1</sub>-C<sub>10</sub> alkyl, -C(O)C<sub>1</sub>-C<sub>10</sub> haloalkyl, -OC(O)C<sub>1</sub>-C<sub>10</sub> alkyl, -SO<sub>2</sub>(C<sub>1</sub>-C<sub>10</sub> alkyl), -SO<sub>2</sub>(phenyl), -SO<sub>2</sub>(C<sub>1</sub>-C<sub>10</sub> haloalkyl), -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NH(C<sub>1</sub>-C<sub>10</sub> alkyl), -SO<sub>2</sub>NH(phenyl), -NHSO<sub>2</sub>(C<sub>1</sub>-C<sub>10</sub> alkyl), -NHSO<sub>2</sub>(phenyl), and -NHSO<sub>2</sub>(C<sub>1</sub>-C<sub>10</sub> haloalkyl).

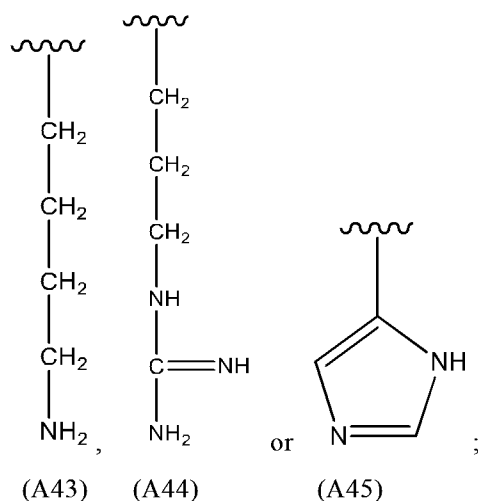
In some embodiments, L<sub>1</sub> may be selected from the group consisting of the groups of Formulae (A1)-(A26) and any combination thereof, wherein the structures and definitions of A1-A26 are as follows:





- wherein each  $j_1$  is independently an integer of 1-20; each  $j_2$  is independently an integer of 1-20;  
 5 each  $R^1$  is independently a  $C_1$ - $C_{10}$  alkyl;  
 each  $R_a$  is independently selected from the group consisting of the groups of Formulae (A27)-(A45) or any combination thereof:





each Rb is independently a C<sub>1</sub>-C<sub>10</sub> alkyl; and

5      ~~~~~ represents the site where a group is covalently linked.

Those skilled in the art would understand that, though L<sub>1</sub> is defined as a linear alkyl for convenience, but it may not be a linear group or be named differently, such as an amine or alkenyl produced by the above replacement and/or substitution. For the purpose of the present disclosure, the length of L<sub>1</sub> is the number of the atoms in the chain linking the two attachment points. For this purpose, a ring obtained by replacing a carbon atom in the linear alkylene, such as a heterocyclylene or heteroarylene, is counted as one atom.

M<sub>1</sub> represents a targeting group, of which the definitions and options are the same as those of the above targeting groups. In some embodiments, each M<sub>1</sub> is independently one selected from the ligands that have affinity to the asialoglycoprotein receptor on the surface of mammalian hepatocytes.

When M<sub>1</sub> is a ligand that has affinity to the asialoglycoprotein receptor on the surface of mammalian hepatocyte, in some embodiments, n<sub>1</sub> may be an integer of 1-3, and n<sub>3</sub> may be an integer of 0-4 to ensure that the number of the M<sub>1</sub> targeting group in the conjugate may be at least 2. In some embodiments,  $n_1 + n_3 \geq 2$ , such that the number of the M<sub>1</sub> targeting group is at least 3, thereby rendering the M<sub>1</sub> targeting group to more easily bind to the asialoglycoprotein receptor on the surface of hepatocytes, which may facilitates the endocytosis of the conjugate into cells. Experiments have shown that when the number of the M<sub>1</sub> ligand is greater than 3, the ease of the binding between the M<sub>1</sub> ligand and the asialoglycoprotein receptor on the surface of hepatocytes is not significantly increased. Therefore, in view of various aspects such as synthesis convenience, structure/process costs and delivery efficiency, in some embodiments, n<sub>1</sub> is an integer of 1-2, n<sub>3</sub> is an integer of 0-1, and  $n_1 + n_3 = 2-3$ .

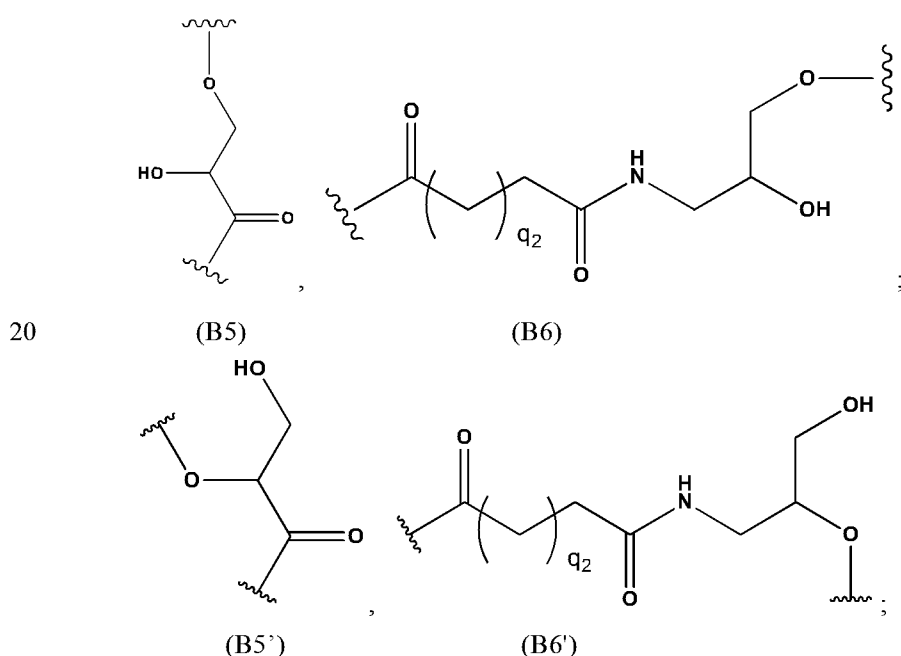
In some embodiments, when each m<sub>1</sub>, m<sub>2</sub>, or m<sub>3</sub> independently of one another is an integer selected from 2-10, the steric positions among many M<sub>1</sub> ligands may be suitable for the binding between the M<sub>1</sub> ligands and the asialoglycoprotein receptor on the surface of hepatocytes. In order to make the conjugate of the present disclosure have simpler structure, easier synthesis and/or reduced cost, in some embodiments, each m<sub>1</sub>, m<sub>2</sub> or m<sub>3</sub> independently of one another is an integer of 2-5; in some embodiments, m<sub>1</sub> = m<sub>2</sub> = m<sub>3</sub>.

Those skilled in the art would understand that when R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub>, R<sub>14</sub>, or R<sub>15</sub> independently of one another is one selected from H, C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>1</sub>-C<sub>10</sub> haloalkyl, and C<sub>1</sub>-C<sub>10</sub> alkoxy, they would not change the properties

of the siRNA conjugate of the present disclosure and could all achieve the purpose of the present disclosure. In some embodiments, R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub>, R<sub>14</sub>, or R<sub>15</sub> independently of one another is selected from H, methyl or ethyl. In some embodiments, R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub>, R<sub>14</sub>, and R<sub>15</sub> are H.

5 R<sub>3</sub> is a group having the structure as shown by Formula (A59), wherein E<sub>1</sub> is OH, SH or BH<sub>2</sub>, and considering  
the easy availability of the starting materials, in some embodiments, E<sub>1</sub> is OH or SH.

R<sub>2</sub> is selected to achieve the linkage between the group as shown by Formula (A59) and the N atom on a nitrogenous backbone. In the context of the present disclosure, a “nitrogenous backbone” refers to a chain structure in which the N atom are coadjacently linked to the carbon atoms to which R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub>, R<sub>14</sub>, and R<sub>15</sub> are attached. Therefore, R<sub>2</sub> may be any linking group capable of linking the group as shown by Formula (A59) to the N atom on the nitrogenous backbone by suitable means. In some embodiments, in the case where the siRNA conjugate as shown by Formula (308) is prepared by a solid phase synthesis process, R<sub>2</sub> group needs to have both a site linking to the N atom on the nitrogenous backbone and a site linking to the P atom in R<sub>3</sub>. In some embodiments, in R<sub>2</sub>, the site linking to the N atom on the nitrogenous backbone forms an amide bond with the N atom, and the site linking to the P atom in R<sub>3</sub> forms a phosphoester bond with the P atom. In some embodiments, R<sub>2</sub> may be B5, B6, B5', or B6';



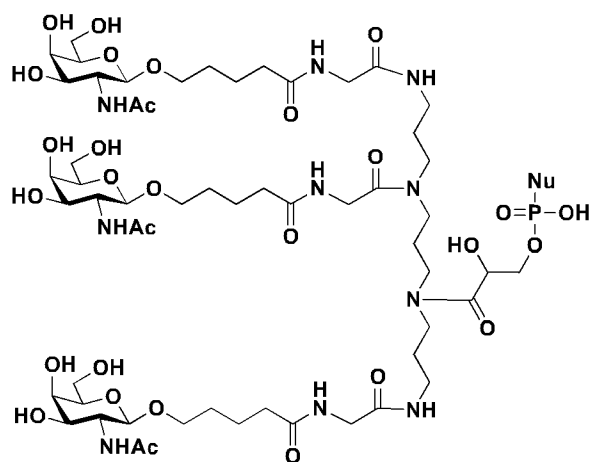
wherein  represents the site where the group is linked via a covalent bond; 25 q<sub>2</sub> is an integer of 1-10; in some embodiments, q<sub>2</sub> is an integer of 1-5.

L<sub>1</sub> is used to link the M<sub>1</sub> targeting group to the N atom on the nitrogenous backbone, thereby providing liver targeting function for the siRNA conjugate as shown by Formula (308). In some embodiments, L<sub>1</sub> is selected from the connection combinations of one or more of Formulae (A1)-(A26). In some embodiments, L<sub>1</sub> is selected from the connection combinations of one or more of Formulae (A1), (A4), (A5), (A6), (A8), (A10), (A11), and (A13). In some embodiments, L<sub>1</sub> is selected from the connection combinations of at least two of Formulae (A1), (A4), (A8), (A10), and (A11). In some embodiments, L<sub>1</sub> is selected from the connection combinations of at least two of Formulae (A1), (A8) and (A10).

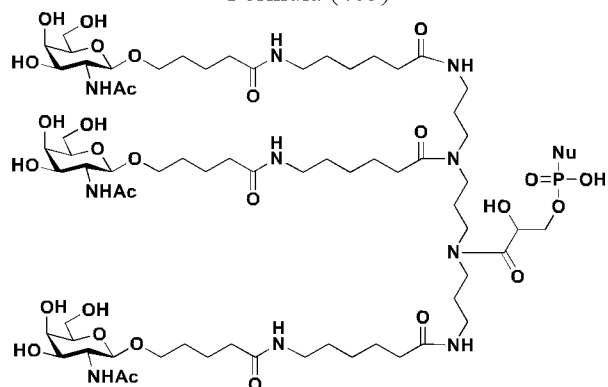
In some embodiments,  $L_1$  may have a length of 3 to 25, 3 to 20, 4 to 15 or 5 to 12 atoms. In some embodiments,  $L_1$  has a length of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, or 60 atoms.

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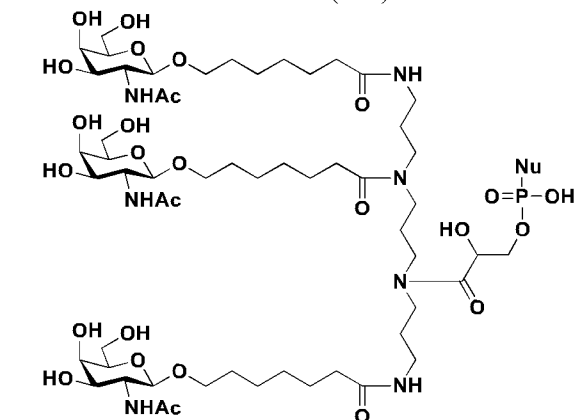
In some embodiments,  $j_1$  is an integer of 2-10, and in some embodiments,  $j_1$  is an integer of 3-5. In some embodiments,  $j_2$  is an integer of 2-10, and in some embodiments,  $j_2$  is an integer of 3-5.  $R'$  is a  $C_1$ - $C_4$  alkyl, and in some embodiments,  $R'$  is one of methyl, ethyl and isopropyl.  $R_a$  is one of Formulae (A27), (A28), (A29), (A30), and (A31), and in some embodiments,  $R_a$  is Formula (A27) or (A28).  $R_b$  is a  $C_1$ - $C_5$  alkyl, and in some  
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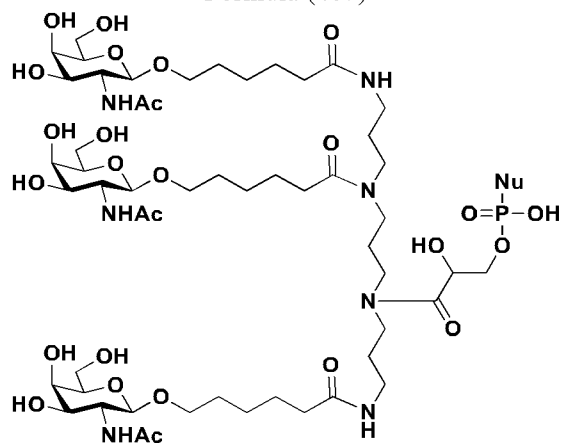
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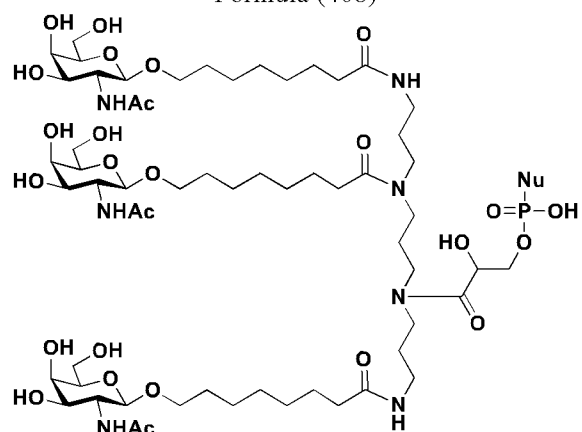
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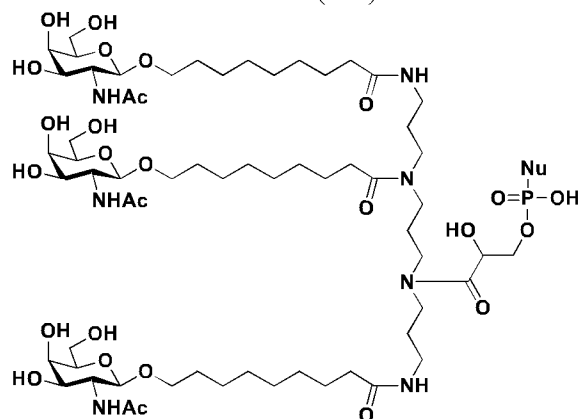
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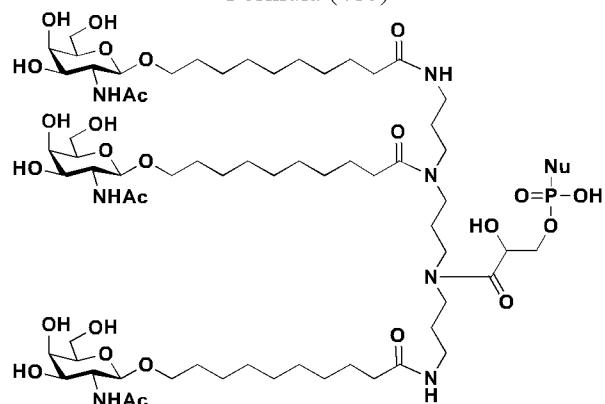
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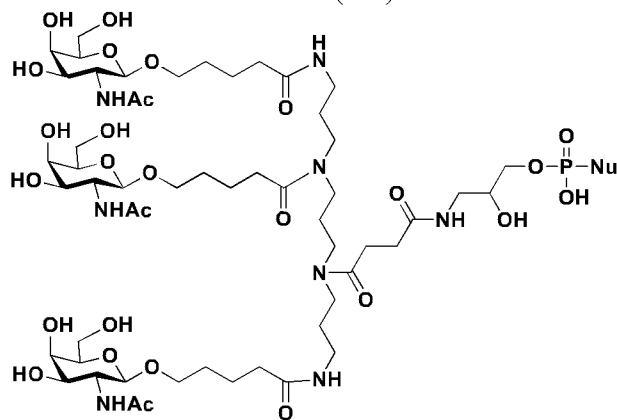
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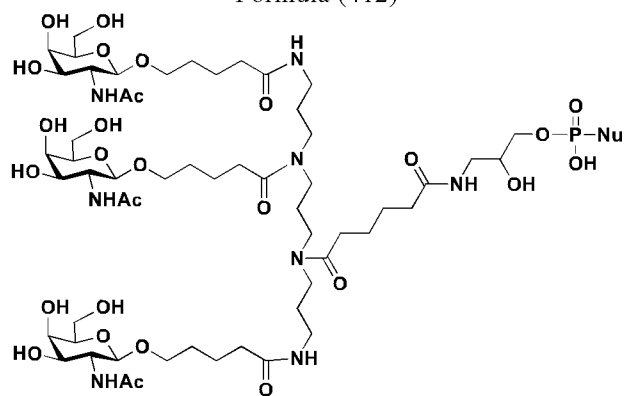
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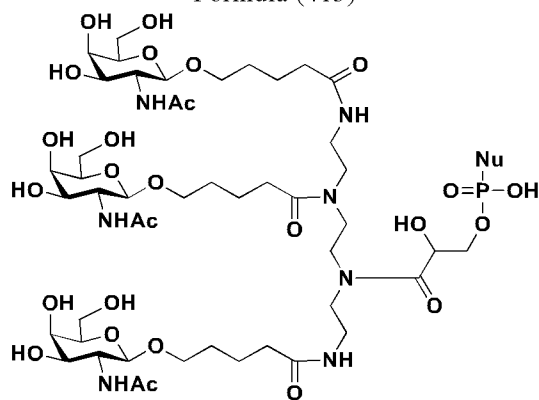
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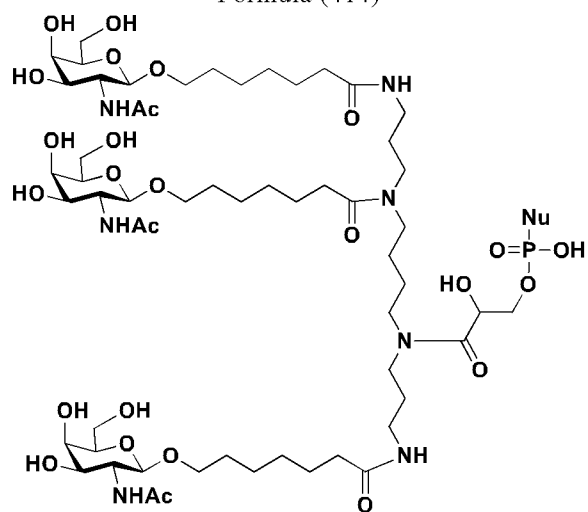
Formula (412)



Formula (413)

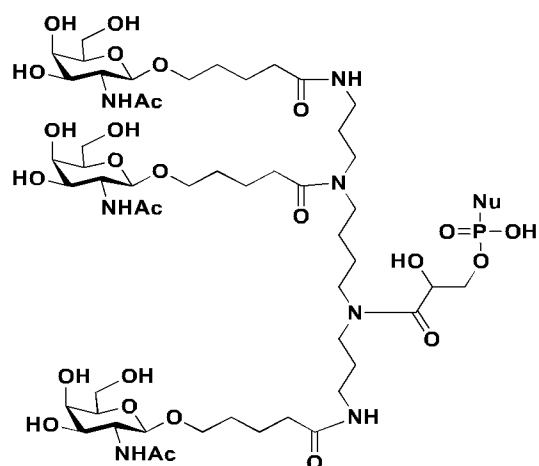


Formula (414)

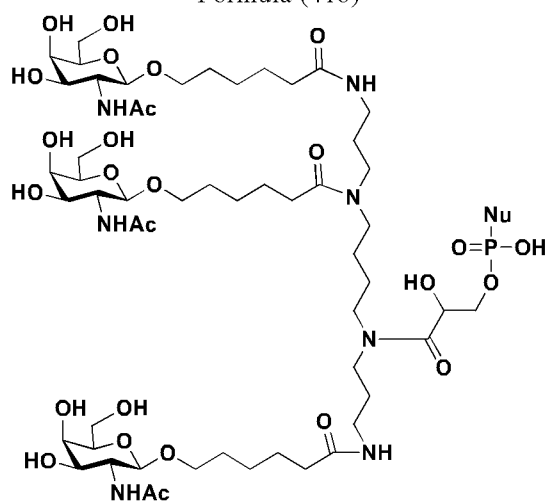


Formula (415)

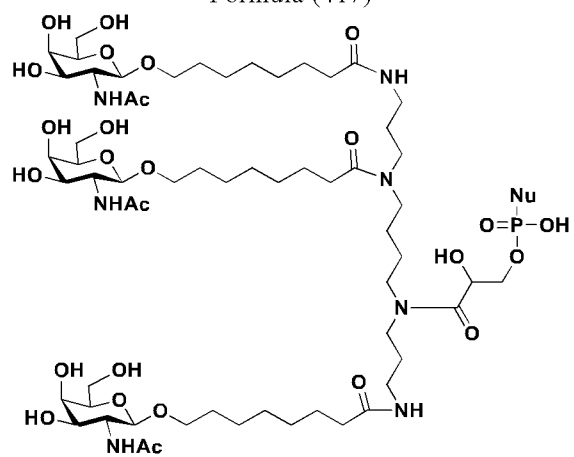
5



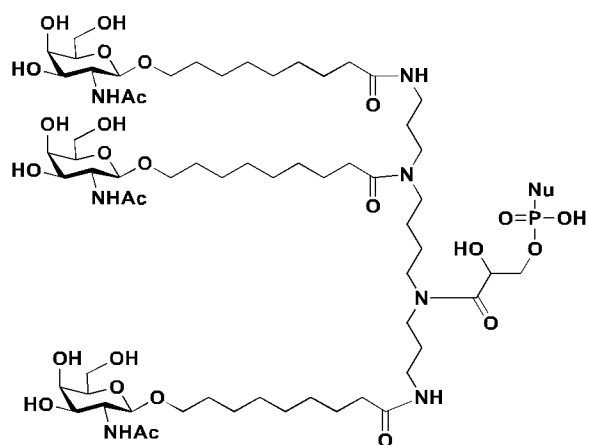
Formula (416)



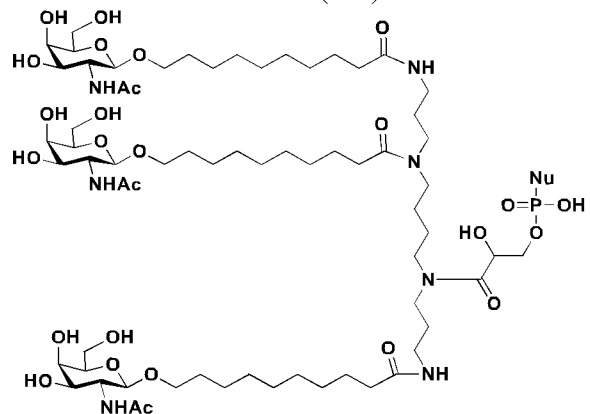
Formula (417)



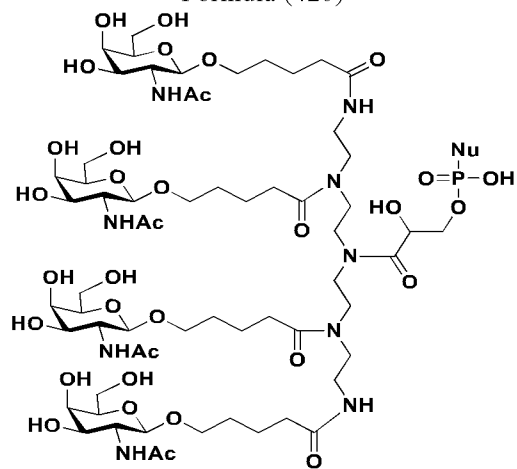
Formula (418)



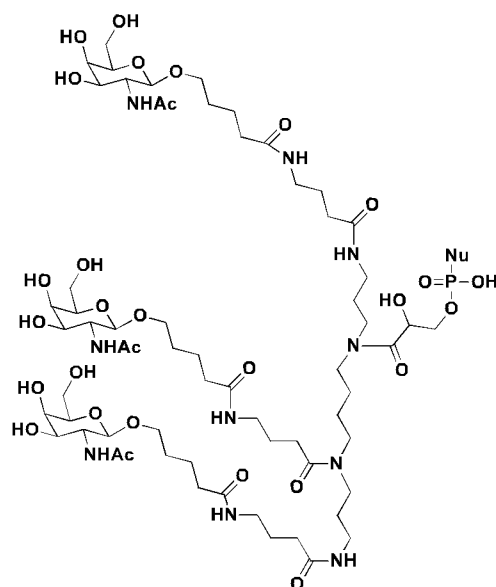
Formula (419)



Formula (420)



Formula (421)



Formula (422).

In some embodiments, the P atom in Formula (A59) may be linked to any possible position in the siRNA sequence. For example, the P atom in Formula (A59) may be linked to any nucleotide in the sense or antisense strand of the siRNA. In some embodiments, the P atom in Formula (A59) is linked to any nucleotide in the sense strand of the siRNA. In some embodiments, the P atom in Formula (A59) may be linked to a terminal region of the sense or antisense strand of the siRNA. In some embodiments, the P atom in Formula (A59) is linked to a terminal region of the sense strand of the siRNA. Said terminal region refers to the first 4 nucleotides counted from one terminal of the sense or antisense strand. In some embodiments, the P atom in Formula (A59) is linked to either terminal of the sense or antisense strand of the siRNA. In some embodiments, the P atom in Formula (A59) is linked to 3' terminal of the sense strand of the siRNA. In the case where the P atom in Formula (A59) is linked to the above position of the sense strand of the siRNA, after having entered into cells, the siRNA conjugate as shown by Formula (308) can release a separate antisense strand of the siRNA during unwinding, thereby blocking the translation of the PCSK9 mRNA into a protein and inhibiting the expression of PCSK9 gene.

In some embodiments, the P atom in Formula (A59) may be linked to any possible position of a nucleotide in the siRNA, for example, position 5', position 2', position 3', or the base of the nucleotide. In some embodiments, the P atom in Formula (A59) may be linked to position 2', 3', or 5' of a nucleotide in the siRNA by forming a phosphodiester bond. In some embodiments, the P atom in Formula (A59) is linked to an oxygen atom formed by dehydrogenation of 3'-hydroxy of the nucleotide at 3' terminal of the sense strand in the siRNA (in this case, the P atom in Formula (A59) may be also regarded as the P atom in the phosphate group of the siRNA), or the P atom in Formula (A59) is linked to a nucleotide by substituting a hydrogen atom in 2'-hydroxy of a nucleotide of the sense strand in the siRNA, or the P atom in Formula (A59) is linked to a nucleotide by substituting a hydrogen atom in 5'-hydroxy of the nucleotide at 5' terminal of the sense strand in the siRNA.

The inventors of the present disclosure have surprisingly found that the siRNA conjugate of the present disclosure exhibits significantly improved stability in plasma and low off-target effect, and exhibits relatively high silencing activity against PCSK9 mRNA, and further shows higher inhibitory effect on blood lipid. In some embodiments, the siRNA of the present disclosure can be any one of the siRNAs shown in Tables 1a-1g. The conjugates comprising these siRNAs exhibit higher silencing activity against PCSK9 mRNA.

**Table 1a The sequences of first siRNAs of the present disclosure**

| siRNA NO.     | SEQ ID NO: | Sequence direction 5'-3'                           |
|---------------|------------|----------------------------------------------------|
| siPCSKa1      | 9          | AAGCAAGCAGACAUUUUAUC                               |
|               | 10         | GAUAAAUGUCUGCUUGCUUGG                              |
| siPCSKa2      | 11         | CCAAGCAAGCAGACAUUUUAUC                             |
|               | 12         | GAUAAAUGUCUGCUUGCUUGGGU                            |
| siPCSKa1-M1   | 13         | AmAmGmCmAmAmGfCfAfGmAmCmAmUmUmUmAmUmCm             |
|               | 14         | GmAfUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmGmGm         |
| siPCSKa1-M2   | 15         | AmAmGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm             |
|               | 16         | GmAfUmAmAmAfUmGfUfCmUmGmCmUfUmGfCmUmUmGmGm         |
| siPCSKa1-M3   | 17         | AmAmGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm             |
|               | 18         | GmAfUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmGmGm         |
| siPCSKa2-M1   | 19         | CmCmAmAmGmCmAmAmGfCfAfGmAmCmAmUmUmUmAmUmCm         |
|               | 20         | GmAfUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmGmGmGmUm     |
| siPCSKa2-M2   | 21         | CmCmAmAmGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm         |
|               | 22         | GmAfUmAmAmAfUmGfUfCmUmGmCmUfUmGfCmUmUmGmGmGmUm     |
| siPCSKa2-M3   | 23         | CmCmAmAmGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm         |
|               | 24         | GmAfUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmGmGmGmUm     |
| siPCSKa1-M1S  | 25         | AmsAmsGmCmAmAmGfCfAfGmAmCmAmUmUmUmAmUmCm           |
|               | 26         | GmsAfsUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmsGmsGm     |
| siPCSKa1-M2S  | 27         | AmsAmsGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm           |
|               | 28         | GmsAfsUmAmAmAfUmGfUfCmUmGmCmUfUmGfCmUmUmsGmsGm     |
| siPCSKa1-M3S  | 29         | AmsAmsGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm           |
|               | 30         | GmsAfsUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmsGmsGm     |
| siPCSKa2-M1S  | 31         | CmsCmsAmAmGmCmAmAmGfCfAfGmAmCmAmUmUmUmAmUmCm       |
|               | 32         | GmsAfsUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmGmGmsGmsUm |
| siPCSKa2-M2S  | 33         | CmsCmsAmAmGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm       |
|               | 34         | GmsAfsUmAmAmAfUmGfUfCmUmGmCmUfUmGfCmUmUmGmGmsGmsUm |
| siPCSKa2-M3S  | 35         | CmsCmsAmAmGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm       |
|               | 36         | GmsAfsUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmGmGmsGmsUm |
| siPCSKa1-M1P1 | 37         | AmAmGmCmAmAmGfCfAfGmAmCmAmUmUmUmAmUmCm             |
|               | 38         | P1GmAfUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmGmGm       |
| siPCSKa1-M2P1 | 39         | AmAmGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm             |
|               | 40         | P1GmAfUmAmAmAfUmGfUfCmUmGmCmUfUmGfCmUmUmGmGm       |
| siPCSKa1-M3P1 | 41         | AmAmGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm             |
|               | 42         | P1GmAfUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmGmGm       |
| siPCSKa2-M1P1 | 43         | CmCmAmAmGmCmAmAmGfCfAfGmAmCmAmUmUmUmAmUmCm         |
|               | 44         | P1GmAfUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmGmGmGmUm   |

|                |    |                                                      |
|----------------|----|------------------------------------------------------|
| siPCSKa2-M2P1  | 45 | CmCmAmAmGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm           |
|                | 46 | P1GmAfUmAmAmAfUmGfUfCmUmGmCmUfUmGfCmUmUmGmGmGmUm     |
| siPCSKa2-M3P1  | 47 | CmCmAmAmGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm           |
|                | 48 | P1GmAfUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmGmGmGmUm     |
| siPCSKa1-M1SP1 | 49 | AmsAmsGmCmAmAmGfCfAfGmAmCmAmUmUmUmAmUmCm             |
|                | 50 | P1GmsAfsUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmsGmsGm     |
| siPCSKa1-M2SP1 | 51 | AmsAmsGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm             |
|                | 52 | P1GmsAfsUmAmAmAfUmGfUfCmUmGmCmUfUmGfCmUmUmsGmsGm     |
| siPCSKa1-M3SP1 | 53 | AmsAmsGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm             |
|                | 54 | P1GmsAfsUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmsGmsGm     |
| siPCSKa2-M1SP1 | 55 | CmsCmsAmAmGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm         |
|                | 56 | P1GmsAfsUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmGmGmsGmsUm |
| siPCSKa2-M2SP1 | 57 | CmsCmsAmAmGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm         |
|                | 58 | P1GmsAfsUmAmAmAfUmGfUfCmUmGmCmUfUmGfCmUmUmGmGmsGmsUm |
| siPCSKa2-M3SP1 | 59 | CmsCmsAmAmGmCmAfAmGfCfAfGmAmCmAmUmUmUmAmUmCm         |
|                | 60 | P1GmsAfsUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmGmGmsGmsUm |

**Table 1b The sequences of second siRNAs of the present disclosure**

| siRNA NO.    | SEQ ID NO: | Sequence direction 5'-3'                       |
|--------------|------------|------------------------------------------------|
| siPCSKb1     | 69         | UUUGUAGCAUUUUUAUUA                             |
|              | 70         | UUAAUAAAAAUGCUACAAAAC                          |
| siPCSKb2     | 71         | GUUUUGUAGCAUUUUUAUUA                           |
|              | 72         | UUAAUAAAAAUGCUACAAAACCC                        |
| siPCSKb1-M1  | 73         | UmUmUmGmUmAmGfCfAfUmUmUmUmAmUmUmAmAm           |
|              | 74         | UmUfAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmAmCm     |
| siPCSKb1-M2  | 75         | UmUmUmGmUfAmGfCfAfUmUmUmUmAmUmUmAmAm           |
|              | 76         | UmUfAmAmUmAfAmAfAfAmUmGfCmUfAmCmAmAmAmAmCm     |
| siPCSKb1-M3  | 77         | UmUmUmGmUfAmGfCfAfUmUmUmUmAmUmUmAmAm           |
|              | 78         | UmUfAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmAmCm     |
| siPCSKb2-M1  | 79         | GmUmUmUmUmGmUmAmGfCfAfUmUmUmUmAmUmUmAmAm       |
|              | 80         | UmUfAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmAmCmCmCm |
| siPCSKb2-M2  | 81         | GmUmUmUmUmGmUfAmGfCfAfUmUmUmUmAmUmUmAmAm       |
|              | 82         | UmUfAmAmUmAfAmAfAfAmUmGfCmUfAmCmAmAmAmAmCmCmCm |
| siPCSKb2-M3  | 83         | GmUmUmUmUmGmUfAmGfCfAfUmUmUmUmAmUmUmAmAm       |
|              | 84         | UmUfAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmAmCmCmCm |
| siPCSKb1-M1S | 85         | UmsUmsUmGmUmAmGfCfAfUmUmUmUmAmUmUmAmAm         |
|              | 86         | UmsUfsAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmsAmsCm |
| siPCSKb1-M2S | 87         | UmsUmsUmGmUfAmGfCfAfUmUmUmUmAmUmUmAmAm         |
|              | 88         | UmsUfsAmAmUmAfAmAfAfAmUmGfCmUfAmCmAmAmAmsAmsCm |

|                    |     |                                                      |
|--------------------|-----|------------------------------------------------------|
| siPCSKb1<br>-M3S   | 89  | UmsUmsUmGmUfAmGfCfAfUmUmUmUmUmAmUmUmAmAm             |
|                    | 90  | UmsUfsAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmsAmsCm       |
| siPCSKb2<br>-M1S   | 91  | GmsUmsUmUmUmGmUmAmGfCfAfUmUmUmUmUmAmUmUmAmAm         |
|                    | 92  | UmsUfsAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmAmCmsCmsCm   |
| siPCSKb2<br>-M2S   | 93  | GmsUmsUmUmUmGmUfAmGfCfAfUmUmUmUmUmAmUmUmAmAm         |
|                    | 94  | UmsUfsAmAmUmAfAmAfAfAmUmGfCmUfAmCmAmAmAmAmCmsCmsCm   |
| siPCSKb2<br>-M3S   | 95  | GmsUmsUmUmUmGmUfAmGfCfAfUmUmUmUmUmAmUmUmAmAm         |
|                    | 96  | UmsUfsAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmAmCmsCmsCm   |
| siPCSKb1<br>-M1P1  | 97  | UmUmUmGmUmAmGfCfAfUmUmUmUmUmAmUmUmAmAm               |
|                    | 98  | P1UmUfAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmAmCm         |
| siPCSKb1<br>-M2P1  | 99  | UmUmUmGmUfAmGfCfAfUmUmUmUmUmAmUmUmAmAm               |
|                    | 100 | P1UmUfAmAmUmAfAmAfAfAmUmGfCmUfAmCmAmAmAmAmCm         |
| siPCSKb1<br>-M3P1  | 101 | UmUmUmGmUfAmGfCfAfUmUmUmUmUmAmUmUmAmAm               |
|                    | 102 | P1UmUfAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmAmCm         |
| siPCSKb2<br>-M1P1  | 103 | GmUmUmUmUmGmUmAmGfCfAfUmUmUmUmUmAmUmUmAmAm           |
|                    | 104 | P1UmUfAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmAmCmCmCm     |
| siPCSKb2<br>-M2P1  | 105 | GmUmUmUmUmGmUfAmGfCfAfUmUmUmUmUmAmUmUmAmAm           |
|                    | 106 | P1UmUfAmAmUmAfAmAfAfAmUmGfCmUfAmCmAmAmAmAmCmCmCm     |
| siPCSKb2<br>-M3P1  | 107 | GmUmUmUmUmGmUfAmGfCfAfUmUmUmUmUmAmUmUmAmAm           |
|                    | 108 | P1UmUfAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmAmCmCmCm     |
| siPCSKb1<br>-M1SP1 | 109 | UmsUmsUmGmUmAmGfCfAfUmUmUmUmUmAmUmUmAmAm             |
|                    | 110 | P1UmsUfsAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmsAmsCm     |
| siPCSKb1<br>-M2SP1 | 111 | UmsUmsUmGmUfAmGfCfAfUmUmUmUmUmAmUmUmAmAm             |
|                    | 112 | P1UmsUfsAmAmUmAfAmAfAfAmUmGfCmUfAmCmAmAmAmsAmsCm     |
| siPCSKb1<br>-M3SP1 | 113 | UmsUmsUmGmUfAmGfCfAfUmUmUmUmUmAmUmUmAmAm             |
|                    | 114 | P1UmsUfsAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmsAmsCm     |
| siPCSKb2<br>-M1SP1 | 115 | GmsUmsUmUmUmGmUmAmGfCfAfUmUmUmUmUmAmUmUmAmAm         |
|                    | 116 | P1UmsUfsAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmAmCmsCmsCm |
| siPCSKb2<br>-M2SP1 | 117 | GmsUmsUmUmUmGmUfAmGfCfAfUmUmUmUmUmAmUmUmAmAm         |
|                    | 118 | P1UmsUfsAmAmUmAfAmAfAfAmUmGfCmUfAmCmAmAmAmAmCmsCmsCm |
| siPCSKb2<br>-M3SP1 | 119 | GmsUmsUmUmUmGmUfAmGfCfAfUmUmUmUmUmAmUmUmAmAm         |
|                    | 120 | P1UmsUfsAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmAmCmsCmsCm |

**Table 1c The sequences of third siRNAs of the present disclosure**

| siRNA<br>NO. | SEQ<br>ID<br>NO: | Sequence direction 5'-3' |
|--------------|------------------|--------------------------|
|--------------|------------------|--------------------------|

|                   |     |                                                    |
|-------------------|-----|----------------------------------------------------|
| siPCSKc1          | 129 | GCCUGGAGUUUAUUCGGAA                                |
|                   | 130 | UUCCGAAUAAACUCCAGGCCU                              |
| siPCSKc2          | 131 | AGGCCUGGAGUUUAUUCGGAA                              |
|                   | 132 | UUCCGAAUAAACUCCAGGCCUAU                            |
| siPCSKc1<br>-M1   | 133 | GmCmCmUmGmGmAfGfUfUmUmAmUmUmCmGmGmAmAm             |
|                   | 134 | UmUfCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmCmUm         |
| siPCSKc1<br>-M2   | 135 | GmCmCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm             |
|                   | 136 | UmUfCmCmGmAfAmUfAfAmAmCmUmCfCmAfGmGmCmCmUm         |
| siPCSKc1<br>-M3   | 137 | GmCmCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm             |
|                   | 138 | UmUfCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmCmUm         |
| siPCSKc2<br>-M1   | 139 | AmGmGmCmCmUmGmGmAfGfUfUmUmAmUmUmCmGmGmAmAm         |
|                   | 140 | UmUfCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmCmUmAmUm     |
| siPCSKc2<br>-M2   | 141 | AmGmGmCmCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm         |
|                   | 142 | UmUfCmCmGmAfAmUfAfAmAmCmUmCfCmAfGmGmCmCmUmAmUm     |
| siPCSKc2<br>-M3   | 143 | AmGmGmCmCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm         |
|                   | 144 | UmUfCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmCmUmAmUm     |
| siPCSKc1<br>-M1S  | 145 | GmsCmsCmUmGmGmAfGfUfUmUmAmUmUmCmGmGmAmAm           |
|                   | 146 | UmsUfsCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmsCmsUm     |
| siPCSKc1<br>-M2S  | 147 | GmsCmsCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm           |
|                   | 148 | UmsUfsCmCmGmAfAmUfAfAmAmCmUmCfCmAfGmGmCmsCmsUm     |
| siPCSKc1<br>-M3S  | 149 | GmsCmsCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm           |
|                   | 150 | UmsUfsCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmsCmsUm     |
| siPCSKc2<br>-M1S  | 151 | AmsGmsGmCmCmUmGmGmAfGfUfUmUmAmUmUmCmGmGmAmAm       |
|                   | 152 | UmsUfsCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmCmUmsAmsUm |
| siPCSKc2<br>-M2S  | 153 | AmsGmsGmCmCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm       |
|                   | 154 | UmsUfsCmCmGmAfAmUfAfAmAmCmUmCfCmAfGmGmCmCmUmsAmsUm |
| siPCSKc2<br>-M3S  | 155 | AmsGmsGmCmCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm       |
|                   | 156 | UmsUfsCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmCmUmsAmsUm |
| siPCSKc1<br>-M1P1 | 157 | GmCmCmUmGmGmAfGfUfUmUmAmUmUmCmGmGmAmAm             |
|                   | 158 | P1UmUfCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmCmUm       |
| siPCSKc1<br>-M2P1 | 159 | GmCmCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm             |
|                   | 160 | P1UmUfCmCmGmAfAmUfAfAmAmCmUmCfCmAfGmGmCmCmUm       |
| siPCSKc1<br>-M3P1 | 161 | GmCmCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm             |
|                   | 162 | P1UmUfCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmCmUm       |
| siPCSKc2<br>-M1P1 | 163 | AmGmGmCmCmUmGmGmAfGfUfUmUmAmUmUmCmGmGmAmAm         |
|                   | 164 | P1UmUfCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmCmUmAmUm   |
| siPCSKc2<br>-M2P1 | 165 | AmGmGmCmCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm         |
|                   | 166 | P1UmUfCmCmGmAfAmUfAfAmAmCmUmCfCmAfGmGmCmCmUmAmUm   |
|                   | 167 | AmGmGmCmCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm         |

|                    |     |                                                      |
|--------------------|-----|------------------------------------------------------|
| siPCSKc2<br>-M3P1  | 168 | P1UmUfCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmCmUmAmUm     |
| siPCSKc1<br>-M1SP1 | 169 | GmsCmsCmUmGmGmAfGfUfUmUmAmUmUmCmGmGmAmAm             |
|                    | 170 | P1UmsUfsCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmsCmsUm     |
| siPCSKc1<br>-M2SP1 | 171 | GmsCmsCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm             |
|                    | 172 | P1UmsUfsCmCmGmAfAmUfAfAmAmCmUmCfCmAfGmGmCmsCmsUm     |
| siPCSKc1<br>-M3SP1 | 173 | GmsCmsCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm             |
|                    | 174 | P1UmsUfsCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmsCmsUm     |
| siPCSKc2<br>-M1SP1 | 175 | AmsGmsGmCmCmUmGmGmAfGfUfUmUmAmUmUmCmGmGmAmAm         |
|                    | 176 | P1UmsUfsCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmCmUmsAmsUm |
| siPCSKc2<br>-M2SP1 | 177 | AmsGmsGmCmCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm         |
|                    | 178 | P1UmsUfsCmCmGmAfAmUfAfAmAmCmUmCfCmAfGmGmCmCmUmsAmsUm |
| siPCSKc2<br>-M3SP1 | 179 | AmsGmsGmCmCmUmGfGmAfGfUfUmUmAmUmUmCmGmGmAmAm         |
|                    | 180 | P1UmsUfsCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmCmUmsAmsUm |

**Table 1d The sequences of fourth siRNAs of the present disclosure**

| siRNA<br>NO. | SEQ<br>ID<br>NO: | Sequence direction 5'-3'                       |
|--------------|------------------|------------------------------------------------|
| siPCSKd1     | 189              | CUGUUUUGCUUUUGUAACU                            |
|              | 190              | AGUUACAAAAGCAAAACAGGU                          |
| siPCSKd2     | 191              | ACCUGUUUUGCUUUUGUAACU                          |
|              | 192              | AGUUACAAAAGCAAAACAGGUCU                        |
| siPCSKd1-M1  | 193              | CmUmGmUmUmUmUfGfCfUmUmUmUmGmUmAmAmCmUm         |
|              | 194              | AmGfUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmGmUm     |
| siPCSKd1-M2  | 195              | CmUmGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm         |
|              | 196              | AmGfUmUmAmCfAmAfAfAmGmCmAmAfAmAfCmAmGmGmUm     |
| siPCSKd1-M3  | 197              | CmUmGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm         |
|              | 198              | AmGfUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmGmUm     |
| siPCSKd2-M1  | 199              | AmCmCmUmGmUmUmUmUfGfCfUmUmUmUmGmUmAmAmCmUm     |
|              | 200              | AmGfUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmGmUmCmUm |
| siPCSKd2-M2  | 201              | AmCmCmUmGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm     |
|              | 202              | AmGfUmUmAmCfAmAfAfAmGmCmAmAfAmAfCmAmGmGmUmCmUm |
| siPCSKd2-M3  | 203              | AmCmCmUmGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm     |
|              | 204              | AmGfUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmGmUmCmUm |
| siPCSKd1-M1S | 205              | CmsUmsGmUmUmUmUfGfCfUmUmUmUmGmUmAmAmCmUm       |
|              | 206              | AmsGfsUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmsGmsUm |
| siPCSKd1-M2S | 207              | CmsUmsGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm       |
|              | 208              | AmsGfsUmUmAmCfAmAfAfAmGmCmAmAfAmAfCmAmGmsGmsUm |
| siPCSKd1-M3S | 209              | CmsUmsGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm       |
|              | 210              | AmsGfsUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmsGmsUm |
|              | 211              | AmsCmsCmUmGmUmUmUmUfGfCfUmUmUmUmGmUmAmAmCmUm   |

|                |     |                                                      |
|----------------|-----|------------------------------------------------------|
| siPCSKd2-M1S   | 212 | AmsGfsUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmGmUmsCmsUm   |
| siPCSKd2-M2S   | 213 | AmsCmsCmUmGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm         |
|                | 214 | AmsGfsUmUmAmCfAmAfAfAmGmCmAmAfAmAfCmAmGmGmUmsCmsUm   |
| siPCSKd2-M3S   | 215 | AmsCmsCmUmGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm         |
|                | 216 | AmsGfsUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmGmUmsCmsUm   |
| siPCSKd1-M1P1  | 217 | CmUmGmUmUmUmUfGfCfUmUmUmUmGmUmAmAmCmUm               |
|                | 218 | P1AmGfUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmGmUm         |
| siPCSKd1-M2P1  | 219 | CmUmGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm               |
|                | 220 | P1AmGfUmUmAmCfAmAfAfAmGmCmAmAfAmAfCmAmGmGmUm         |
| siPCSKd1-M3P1  | 221 | CmUmGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm               |
|                | 222 | P1AmGfUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmGmUm         |
| siPCSKd2-M1P1  | 223 | AmCmCmUmGmUmUmUmUfGfCfUmUmUmUmGmUmAmAmCmUm           |
|                | 224 | P1AmGfUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmGmUmCmUm     |
| siPCSKd2-M2P1  | 225 | AmCmCmUmGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm           |
|                | 226 | P1AmGfUmUmAmCfAmAfAfAmGmCmAmAfAmAfCmAmGmGmUmCmUm     |
| siPCSKd2-M3P1  | 227 | AmCmCmUmGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm           |
|                | 228 | P1AmGfUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmGmUmCmUm     |
| siPCSKd1-M1SP1 | 229 | CmsUmsGmUmUmUmUfGfCfUmUmUmUmGmUmAmAmCmUm             |
|                | 230 | P1AmsGfsUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmsGmsUm     |
| siPCSKd1-M2SP1 | 231 | CmsUmsGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm             |
|                | 232 | P1AmsGfsUmUmAmCfAmAfAfAmGmCmAmAfAmAfCmAmGmsGmsUm     |
| siPCSKd1-M3SP1 | 233 | CmsUmsGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm             |
|                | 234 | P1AmsGfsUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmsGmsUm     |
| siPCSKd2-M1SP1 | 235 | AmsCmsCmUmGmUmUmUmUfGfCfUmUmUmUmGmUmAmAmCmUm         |
|                | 236 | P1AmsGfsUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmGmUmsCmsUm |
| siPCSKd2-M2SP1 | 237 | AmsCmsCmUmGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm         |
|                | 238 | P1AmsGfsUmUmAmCfAmAfAfAmGmCmAmAfAmAfCmAmGmGmUmsCmsUm |
| siPCSKd2-M3SP1 | 239 | AmsCmsCmUmGmUmUfUmUfGfCfUmUmUmUmGmUmAmAmCmUm         |
|                | 240 | P1AmsGfsUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmGmUmsCmsUm |

**Table 1e The sequences of fifth siRNAs of the present disclosure**

| siRNA NO. | SEQ ID NO: | Sequence direction 5'-3' |
|-----------|------------|--------------------------|
| siPCSKe1  | 249        | GGUUUUGUAGCAUUUUUAU      |
|           | 250        | AUAAAAAUGCUACAAAACCCA    |
| siPCSKe2  | 251        | UGGGUUUUGUAGCAUUUUUAU    |

|                |     |                                                      |
|----------------|-----|------------------------------------------------------|
|                | 252 | AUAAAAAUGCUACAAAACCCAGA                              |
| siPCSKe1-M1    | 253 | GmGmUmUmUmUmGfUfAfGmCmAmUmUmUmUmUmAmUm               |
|                | 254 | AmUfAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmCmAm           |
| siPCSKe1-M2    | 255 | GmGmUmUmUfUmGfUfAfGmCmAmUmUmUmUmUmAmUm               |
|                | 256 | AmUfAmAmAmAfAmUfGfCmUmAmCmAfAmAfAmCmCmCmAm           |
| siPCSKe1-M3    | 257 | GmGmUmUmUfUmGfUfAfGmCmAmUmUmUmUmUmAmUm               |
|                | 258 | AmUfAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmCmAm           |
| siPCSKe2-M1    | 259 | UmGmGmGmUmUmUmUmGfUfAfGmCmAmUmUmUmUmUmAmUm           |
|                | 260 | AmUfAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmCmAmGmAm       |
| siPCSKe2-M2    | 261 | UmGmGmGmUmUmUfUmGfUfAfGmCmAmUmUmUmUmUmAmUm           |
|                | 262 | AmUfAmAmAmAfAmUfGfCmUmAmCmAfAmAfAmCmCmCmAmGmAm       |
| siPCSKe2-M3    | 263 | UmGmGmGmUmUmUfUmGfUfAfGmCmAmUmUmUmUmUmAmUm           |
|                | 264 | AmUfAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmCmAmGmAm       |
| siPCSKe1-M1S   | 265 | GmsGmsUmUmUmUmGfUfAfGmCmAmUmUmUmUmUmAmUm             |
|                | 266 | AmsUfsAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmsCmsAm       |
| siPCSKe1-M2S   | 267 | GmsGmsUmUmUfUmGfUfAfGmCmAmUmUmUmUmUmAmUm             |
|                | 268 | AmsUfsAmAmAmAfAmUfGfCmUmAmCmAfAmAfAmCmCmsCmsAm       |
| siPCSKe1-M3S   | 269 | GmsGmsUmUmUfUmGfUfAfGmCmAmUmUmUmUmUmAmUm             |
|                | 270 | AmsUfsAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmsCmsAm       |
| siPCSKe2-M1S   | 271 | UmsGmsGmGmUmUmUmUmGfUfAfGmCmAmUmUmUmUmUmAmUm         |
|                | 272 | AmsUfsAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmCmAmsGmsAm   |
| siPCSKe2-M2S   | 273 | UmsGmsGmGmUmUmUfUmGfUfAfGmCmAmUmUmUmUmUmAmUm         |
|                | 274 | AmsUfsAmAmAmAfAmUfGfCmUmAmCmAfAmAfAmCmCmCmAmsGmsAm   |
| siPCSKe2-M3S   | 275 | UmsGmsGmGmUmUmUfUmGfUfAfGmCmAmUmUmUmUmUmAmUm         |
|                | 276 | AmsUfsAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmCmAmsGmsAm   |
| siPCSKe1-M1P1  | 277 | GmGmUmUmUmUmGfUfAfGmCmAmUmUmUmUmUmAmUm               |
|                | 278 | P1AmUfAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmCmAm         |
| siPCSKe1-M2P1  | 279 | GmGmUmUmUfUmGfUfAfGmCmAmUmUmUmUmUmAmUm               |
|                | 280 | P1AmUfAmAmAmAfAmUfGfCmUmAmCmAfAmAfAmCmCmCmAm         |
| siPCSKe1-M3P1  | 281 | GmGmUmUmUfUmGfUfAfGmCmAmUmUmUmUmUmAmUm               |
|                | 282 | P1AmUfAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmCmAm         |
| siPCSKe2-M1P1  | 283 | UmGmGmGmUmUmUmUmGfUfAfGmCmAmUmUmUmUmUmAmUm           |
|                | 284 | P1AmUfAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmCmAmGmA<br>m |
| siPCSKe2-M2P1  | 285 | UmGmGmGmUmUmUfUmGfUfAfGmCmAmUmUmUmUmUmAmUm           |
|                | 286 | P1AmUfAmAmAmAfAmUfGfCmUmAmCmAfAmAfAmCmCmCmAmGmAm     |
| siPCSKe2-M3P1  | 287 | UmGmGmGmUmUmUfUmGfUfAfGmCmAmUmUmUmUmUmAmUm           |
|                | 288 | P1AmUfAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmCmAmGmA<br>m |
| siPCSKe1-M1SP1 | 289 | GmsGmsUmUmUmUmGfUfAfGmCmAmUmUmUmUmUmAmUm             |
|                | 290 | P1AmsUfsAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmsCmsAm     |

|                |     |                                                    |
|----------------|-----|----------------------------------------------------|
| siPCSKe1-M2SP1 | 291 | GmsGmsUmUmUfUmGfUfAfGmCmAmUmUmUmUmAmUm             |
|                | 292 | P1AmsUfsAmAmAmAfAmUfGfCmUmAmCmAfAmAfAmCmCmsCmsAm   |
| siPCSKe1-M3SP1 | 293 | GmsGmsUmUmUfUmGfUfAfGmCmAmUmUmUmUmAmUm             |
|                | 294 | P1AmsUfsAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmsCmsAm   |
| siPCSKe2-M1SP1 | 295 | UmsGmsGmGmUmUmUmUmGfUfAfGmCmAmUmUmUmUmAmUm         |
|                | 296 | P1AmsUfsAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmCmsGmsAm |
| siPCSKe2-M2SP1 | 297 | UmsGmsGmGmUmUmUfUmGfUfAfGmCmAmUmUmUmUmAmUm         |
|                | 298 | P1AmsUfsAmAmAmAfAmUfGfCmUmAmCmAfAmAfAmCmCmCmsGmsAm |
| siPCSKe2-M3SP1 | 299 | UmsGmsGmGmUmUmUfUmGfUfAfGmCmAmUmUmUmUmAmUm         |
|                | 300 | P1AmsUfsAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmCmsGmsAm |

**Table 1f The sequences of sixth siRNAs of the present disclosure**

| siRNA NO.    | SEQ ID NO: | Sequence direction 5'-3'                         |
|--------------|------------|--------------------------------------------------|
| siPCSKf1     | 309        | GUGACUUUUUAAAAUAAAA                              |
|              | 310        | UUUUUUUUUAAAAAGUCACCA                            |
| siPCSKf2     | 311        | UGGUGACUUUUUAAAAUAAAA                            |
|              | 312        | UUUUUUUUUAAAAAGUCACCAUA                          |
| siPCSKf1-M1  | 313        | GmUmGmAmCmUmUfUfUfUmAmAmAmAmUmAmAmAmAm           |
|              | 314        | UmUfUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmCmAm       |
| siPCSKf1-M2  | 315        | GmUmGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm           |
|              | 316        | UmUfUmUmAmUfUmUfUfAmAmAmAmAfGmUfCmAmCmCmAm       |
| siPCSKf1-M3  | 317        | GmUmGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm           |
|              | 318        | UmUfUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmCmAm       |
| siPCSKf2-M1  | 319        | UmGmGmUmGmAmCmUmUfUfUfUmAmAmAmAmUmAmAmAmAm       |
|              | 320        | UmUfUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmCmAmUmAm   |
| siPCSKf2-M2  | 321        | UmGmGmUmGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm       |
|              | 322        | UmUfUmUmAmUfUmUfUfAmAmAmAmAfGmUfCmAmCmCmAmUmAm   |
| siPCSKf2-M3  | 323        | UmGmGmUmGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm       |
|              | 324        | UmUfUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmCmAmUmAm   |
| siPCSKf1-M1S | 325        | GmsUmsGmAmCmUmUfUfUfUmAmAmAmAmUmAmAmAmAm         |
|              | 326        | UmsUfsUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmsCmsAm   |
| siPCSKf1-M2S | 327        | GmsUmsGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm         |
|              | 328        | UmsUfsUmUmAmUfUmUfUfAmAmAmAmAfGmUfCmAmCmsCmsAm   |
| siPCSKf1-M3S | 329        | GmsUmsGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm         |
|              | 330        | UmsUfsUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmsCmsAm   |
| siPCSKf2-M1S | 331        | UmsGmsGmUmGmAmCmUmUfUfUfUmAmAmAmAmUmAmAmAmAm     |
|              | 332        | UmsUfsUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmCmsUmsAm |
|              | 333        | UmsGmsGmUmGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm     |

|                |     |                                                      |
|----------------|-----|------------------------------------------------------|
| siPCSKf2-M2S   | 334 | UmsUfsUmUmAmUfUmUfUfAmAmAmAmAfGmUfCmAmCmCmAmsUmsAm   |
| siPCSKf2-M3S   | 335 | UmsGmsGmUmGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm         |
|                | 336 | UmsUfsUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmCmAmsUmsAm   |
| siPCSKf1-M1P1  | 337 | GmUmGmAmCmUmUfUfUfUmAmAmAmAmUmAmAmAmAm               |
|                | 338 | P1UmUfUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmCmAm         |
| siPCSKf1-M2P1  | 339 | GmUmGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm               |
|                | 340 | P1UmUfUmUmAmUfUmUfUfAmAmAmAmAfGmUfCmAmCmCmAm         |
| siPCSKf1-M3P1  | 341 | GmUmGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm               |
|                | 342 | P1UmUfUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmCmAm         |
| siPCSKf2-M1P1  | 343 | UmGmGmUmGmAmCmUmUfUfUfUmAmAmAmAmUmAmAmAmAm           |
|                | 344 | P1UmUfUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmCmAmUmAm     |
| siPCSKf2-M2P1  | 345 | UmGmGmUmGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm           |
|                | 346 | P1UmUfUmUmAmUfUmUfUfAmAmAmAmAfGmUfCmAmCmCmAmUmAm     |
| siPCSKf2-M3P1  | 347 | UmGmGmUmGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm           |
|                | 348 | P1UmUfUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmCmAmUmAm     |
| siPCSKf1-M1SP1 | 349 | GmsUmsGmAmCmUmUfUfUfUmAmAmAmAmUmAmAmAmAm             |
|                | 350 | P1UmsUfsUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmsCmsAm     |
| siPCSKf1-M2SP1 | 351 | GmsUmsGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm             |
|                | 352 | P1UmsUfsUmUmAmUfUmUfUfAmAmAmAmAfGmUfCmAmCmsCmsAm     |
| siPCSKf1-M3SP1 | 353 | GmsUmsGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm             |
|                | 354 | P1UmsUfsUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmsCmsAm     |
| siPCSKf2-M1SP1 | 355 | UmsGmsGmUmGmAmCmUmUfUfUfUmAmAmAmAmUmAmAmAmAm         |
|                | 356 | P1UmsUfsUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmCmAmsUmsAm |
| siPCSKf2-M2SP1 | 357 | UmsGmsGmUmGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm         |
|                | 358 | P1UmsUfsUmUmAmUfUmUfUfAmAmAmAmAfGmUfCmAmCmCmAmsUmsAm |
| siPCSKf2-M3SP1 | 359 | UmsGmsGmUmGmAmCfUmUfUfUfUmAmAmAmAmUmAmAmAmAm         |
|                | 360 | P1UmsUfsUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmCmAmsUmsAm |

**Table 1g The sequences of seventh siRNAs of the present disclosure**

| siRNA NO. | SEQ ID NO: | Sequence direction 5'-3' |
|-----------|------------|--------------------------|
| siPCSKg3  | 407        | AGACCUGUdTUUGCUUUUGU     |
|           | 408        | ACAAAAGCAAAACAGGUCUAG    |
| siPCSKg4  | 409        | CUAGACCUGUdTUUGCUUUUGU   |
|           | 410        | ACAAAAGCAAAACAGGUCUAGAA  |

|                |     |                                                      |
|----------------|-----|------------------------------------------------------|
| siPCSKg3-M4    | 411 | AmGmAmCmCfUmGfUmdTUmUmGmCmUmUmUmUmGmUm               |
|                | 412 | AmCfAmAfAfAfGmCfAmAfAmAmCmAfGmGfUmCfUmAmGm           |
| siPCSKg4-M5    | 413 | CmUmAmGmAmCmCfUmGfUmdTUmUmGmCmUmUmUmUmGmUm           |
|                | 414 | AmCfAmAfAfAfGmCfAmAfAmAmCmAfGmGfUmCfUmAmGmAmAm       |
| siPCSKg3-M4S   | 415 | AmsGmsAmCmCfUmGfUmdTUmUmGmCmUmUmUmUmGmUm             |
|                | 416 | AmsCfsAmAfAfAfGmCfAmAfAmAmCmAfGmGfUmCfUmsAmsGm       |
| siPCSKg4-M5S   | 417 | CmsUmsAmGmAmCmCfUmGfUmdTUmUmGmCmUmUmUmUmGmUm         |
|                | 418 | AmsCfsAmAfAfAfGmCfAmAfAmAmCmAfGmGfUmCfUmAmGmsAmsAm   |
| siPCSKg3-M4P1  | 419 | AmGmAmCmCfUmGfUmdTUmUmGmCmUmUmUmUmGmUm               |
|                | 420 | P1AmCfAmAfAfAfGmCfAmAfAmAmCmAfGmGfUmCfUmAmGm         |
| siPCSKg4-M5P1  | 421 | CmUmAmGmAmCmCfUmGfUmdTUmUmGmCmUmUmUmUmGmUm           |
|                | 422 | P1AmCfAmAfAfAfGmCfAmAfAmAmCmAfGmGfUmCfUmAmGmAmAm     |
| siPCSKg3-M4SP1 | 423 | AmsGmsAmCmCfUmGfUmdTUmUmGmCmUmUmUmUmGmUm             |
|                | 424 | P1AmsCfsAmAfAfAfGmCfAmAfAmAmCmAfGmGfUmCfUmsAmsGm     |
| siPCSKg4-M5SP1 | 425 | CmsUmsAmGmAmCmCfUmGfUmdTUmUmGmCmUmUmUmUmGmUm         |
|                | 426 | P1AmsCfsAmAfAfAfGmCfAmAfAmAmCmAfGmGfUmCfUmAmGmsAmsAm |

In the siRNA or siRNA conjugate of the present disclosure, each pair of adjacent nucleotides is linked via a phosphodiester bond or phosphorothioate diester bond. The non-bridging oxygen or sulfur atom in the phosphodiester bond or phosphorothioate diester bond is negatively charged, and may be present in the form of hydroxy or sulfhydryl. Moreover, the hydrogen ion in the hydroxy or sulfhydryl may be partially or completely substituted with a cation. The cation may be any cation, such as a metal cation, an ammonium cation  $\text{NH}_4^+$  or an organic ammonium cation. In order to increase solubility, in one embodiment, the cation is one or more selected from an alkali metal cation, an ammonium cation formed by a tertiary amine and a quaternary ammonium cation. The alkali metal ion may be  $\text{K}^+$  and/or  $\text{Na}^+$ , and the cation formed by a tertiary amine may be an ammonium cation formed by triethylamine and/or an ammonium cation formed by N,N-diisopropylethylamine. Thus, the siRNA and the siRNA conjugate of the present disclosure can be at least partially present in the form of salt. In one embodiment, the non-bridging oxygen atom or sulfur atom in the phosphodiester bond or phosphorothioate diester bond at least partly binds to sodium ion, and thus the siRNA and the siRNA conjugate of the present disclosure are present or partially present in the form of sodium salt.

Those skilled in the art clearly know that a modified nucleotide group can be introduced into the siRNA of the present disclosure by a nucleoside monomer with a corresponding modification. The methods for preparing a nucleoside monomer having the corresponding modification and the methods for introducing a modified nucleotide into an siRNA are also well-known to those skilled in the art. All modified nucleoside monomers may be either commercially available or prepared by known methods.

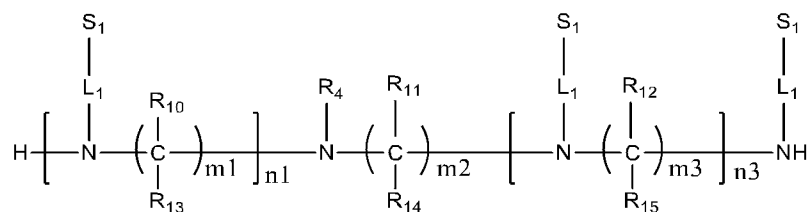
#### **Preparation of the siRNA conjugate as shown by Formula (308)**

The siRNA conjugate as shown by Formula (308) can be prepared by any appropriate synthetic routes.

In some embodiments, the siRNA conjugate as shown by Formula (308) can be prepared by the following method, comprising: sequentially linking nucleoside monomers in 3' to 5' direction according to the type and

sequence of the nucleotides in the sense strand and antisense strands of the siRNA respectively, under the condition for phosphoramidite solid phase synthesis, wherein the step of linking each nucleoside monomer includes a four-step reaction of deprotection, coupling, capping, and oxidation or sulfurization; isolating the sense strand and the antisense strand of the siRNA; and annealing; wherein the siRNA is the above siRNA of the present disclosure.

Moreover, the method further comprises: contacting the compound as shown by Formula (321) with a nucleoside monomer or a nucleotide sequence attached to a solid phase support under coupling reaction condition and in the presence of a coupling agent, thereby linking the compound as shown by Formula (321) to the nucleotide sequence via a coupling reaction. Hereinafter, the compound as shown by Formula (321) is also referred to as a conjugation molecule.



Formula (321)

wherein,

$\text{R}_4$  is a group capable of binding to the siRNA represented by Nu in the compound as shown by Formula (308). In some embodiments,  $\text{R}_4$  is a group capable of binding to the siRNA represented by Nu via a covalent bond. In some embodiments,  $\text{R}_4$  is a group comprising any functional group that may be conjugated to the siRNA represented by Nu via a phosphodiester bond by a reaction;

each  $\text{S}_1$  is independently a group formed by substituting all active hydroxyls in  $\text{M}_1$  with the group  $\text{YCOO-}$ , wherein each Y is independently one selected from the group consisting of methyl, trifluoromethyl, difluoromethyl, monofluoromethyl, trichloromethyl, dichloromethyl, monochloromethyl, ethyl, n-propyl, isopropyl, phenyl, halophenyl, and alkylphenyl; in some embodiments, Y is methyl.

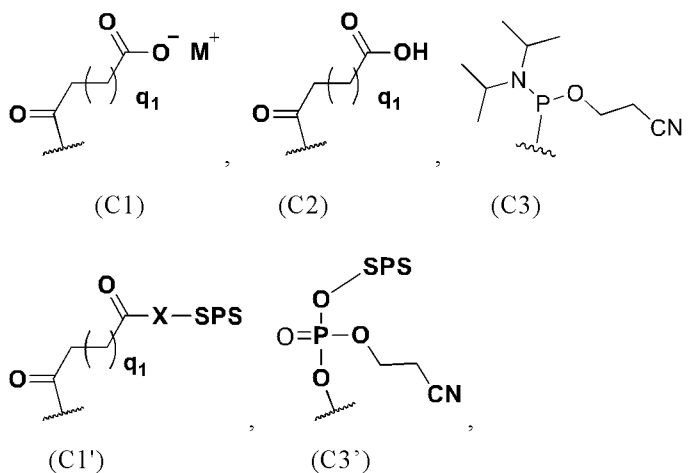
The definitions and options of  $n1$ ,  $n3$ ,  $m1$ ,  $m2$ ,  $m3$ ,  $\text{R}_{10}$ ,  $\text{R}_{11}$ ,  $\text{R}_{12}$ ,  $\text{R}_{13}$ ,  $\text{R}_{14}$ ,  $\text{R}_{15}$ ,  $\text{L}_1$ , and  $\text{M}_1$  are respectively as described above.

$\text{R}_4$  is selected to achieve the linkage to the N atom on a nitrogenous backbone and to provide a suitable reaction site for synthesizing the siRNA conjugate as shown by Formula (308). In some embodiments,  $\text{R}_4$  comprises a  $\text{R}_2$  linking group or a protected  $\text{R}_2$  linking group, and a functional group than can react with an siRNA to form a structure as shown by Formula (A59).

In some embodiments,  $\text{R}_4$  comprises a first functional group that can react with a group on the siRNA represented by Nu or a nucleoside monomer to form a phosphite ester, and a second functional group that can react with a hydroxy group or an amino group to form a covalent bond with, or comprises a solid phase support linked by the covalent bond. In some embodiments, the first functional group is a phosphoramidite, a hydroxy or a protected hydroxy. In some embodiments, the second functional group is a phosphoramidite, a carboxyl or a carboxylate salt. In some embodiments, the second functional group is a solid phase support linked to the rest of the molecule via a covalent bond which is formed by a hydroxy group or an amino group. In some

embodiments, the solid phase support is linked via a phosphoester bond, a carboxyl ester bond or an amide bond. In some embodiments, the solid phase support is a resin.

In some embodiments, the first functional group comprises hydroxy,  $-OR_k$  or a group as shown by Formula (C3); the second functional group comprises a group as shown by Formula (C1), (C2), (C3), (C1'), or (C3'):



wherein  $q_1$  is an integer of 1-4, X is O or NH,  $M^+$  is a cation,  $R_k$  is a hydroxy protecting group, SPS represents a solid phase support, and  $\sim$  represents the site where a group is covalently attached.

In some embodiments, the first functional group comprises a phosphoramidite group, such as the group as shown by Formula (C3). The phosphoramidite group can form a phosphite ester with a hydroxy at any position on a nucleotide (such as a 2'-hydroxy or 3'-hydroxy) by a coupling reaction, and the phosphite ester can form a phosphodiester bond or phosphorothioate ester bond as shown by Formula (A59) via oxidation or sulfurization, so as to conjugate the conjugation molecule to an siRNA. Here, even if the second functional group does not exist, the compound as shown by Formula (321) could still be conjugated to the nucleotide, while not affecting the obtaining of the siRNA conjugate as shown by Formula (308). Under such circumstances, after obtaining a sense or antisense strand of the siRNA by a method such as phosphoramidite solid phase synthesis, the compound as shown by Formula (321) is reacted with a hydroxy on the nucleotide at the terminal of the nucleotide sequence, and a phosphodiester bond linkage or a phosphorothioate bond linkage is formed in the subsequent oxidation or sulfurization process, thereby conjugating the compound as shown by Formula (321) to the siRNA.

In some embodiments, the first functional group comprises a protected hydroxy. In some embodiments, the second functional group comprises a group that can react with a solid phase support to provide a conjugation molecule comprising a solid phase support. In some embodiments, the second functional group comprises a carboxyl, a carboxylate salt or a phosphoramidite, such as the functional group as shown by Formula (C1), (C2) or (C3). When the second functional group comprises a carboxyl or a carboxylate salt, the compound of Formula (321) can react with a hydroxy or an amino group on a solid phase support (such as a resin) via esterification or amidation reaction, to form a conjugation molecule comprising a solid phase support linked via a carboxylate ester bond. When the second functional group comprises a phosphoramidite functional group, the compound of Formula (321) can couple with a hydroxy group on a universal solid phase support (such as a resin), and form a conjugation molecule comprising a solid phase support linked via a phosphodiester bond by oxidation. Next, starting from the above product linked to a solid phase support, the nucleoside monomers

are linked sequentially through a phosphoramidite solid phase synthesis method, so as to obtain a sense strand or an antisense strand of the siRNA linked to a conjugation group. In the process of phosphoramidite solid phase synthesis, the first functional group is deprotected, and then coupled with a phosphoramidite group on a nucleoside monomer under coupling reaction condition.

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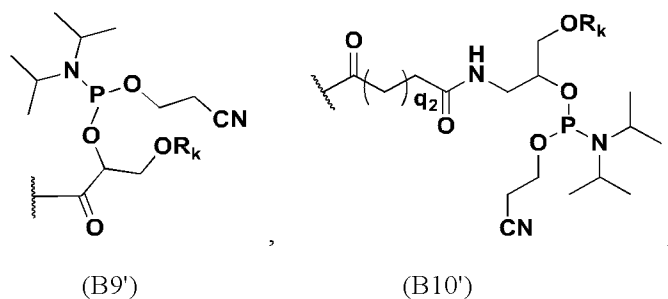
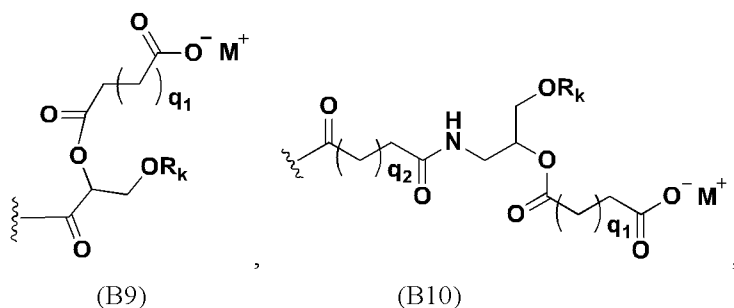
In some embodiments, the first functional group comprises a hydroxy or a protected hydroxy group; the second functional group comprises a solid phase support linked via a carboxylate ester bond, an amide bond, or a phosphoester bond, as shown by Formula (C1') or (C3'). In this case, starting from the compound of Formula (321) in place of a solid phase support, the nucleoside monomers are linked sequentially through a phosphoramidite solid phase synthesis method, so as to obtain a sense strand or an antisense strand of the siRNA linked to a conjugation group.

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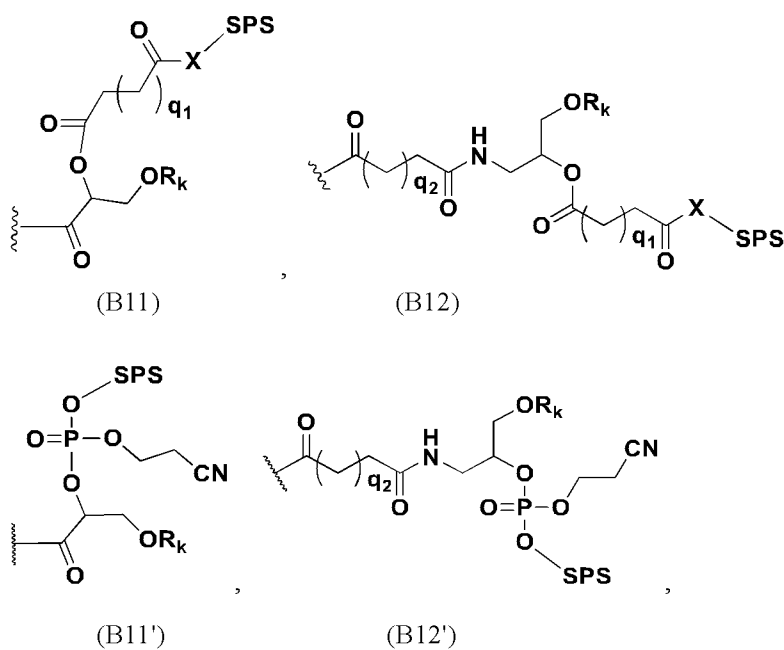
In some embodiments, the carboxylate may be expressed as  $\text{-COO}^-\text{M}^+$ , wherein  $\text{M}^+$  is a cation such as one selected from a metal cation, an ammonium cation  $\text{NH}_4^+$  and an organic ammonium cation. In one embodiment, the metal cation may be an alkali metal cation, such as  $\text{K}^+$  or  $\text{Na}^+$ . In order to increase solubility and facilitate the reaction, in some embodiments, the organic ammonium cation is an ammonium cation formed by a tertiary amine or a quaternary ammonium cation, such as an ammonium cation formed by triethylamine or an ammonium cation formed by N,N-diisopropylethylamine. In some embodiments, the carboxylate is a triethylamine carboxylate or an N,N-diisopropylethylamine carboxylate.

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In some embodiments,  $\text{R}_4$  comprises the structure as shown by Formula (B9), (B10), (B9'), (B10'), (B11), (B12), (B11'), or B(12'):



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wherein  $q_1$  is an integer of 1-4,  $q_2$  is an integer of 1-10, X is O or NH,  $M^+$  is a cation,  $R_k$  is a hydroxy protecting group, SPS represents a solid phase support, and  represents the site where the group is covalently linked. In some embodiments,  $q_1$  is 1 or 2. In some embodiments,  $q_2$  is an integer of 1-5. In some embodiments,  $R_k$  comprises a structure as shown by Formula (B9) or (B10). In some embodiments,  $R_k$  comprises a structure as shown by Formula (B11) or (B12).

10

In some embodiments,  $R_k$  is one or more of Tr (trityl), MMTr (4-methoxytrityl), DMTr (4,4'-dimethoxytrityl), and TMTr (4,4',4''-trimethoxytrityl). In some embodiments,  $R_k$  may be DMTr, i.e., 4,4'-dimethoxytrityl.

15 The definition of  $L_1$  is as described above.

In some embodiments,  $L_1$  is used to link the  $M_1$  targeting group to the N atom on the nitrogenous backbone, thereby providing liver targeting function for the siRNA conjugate as shown by Formula (308). In some embodiments,  $L_1$  comprises any one of Formulae (A1)-(A26), and any combination thereof.

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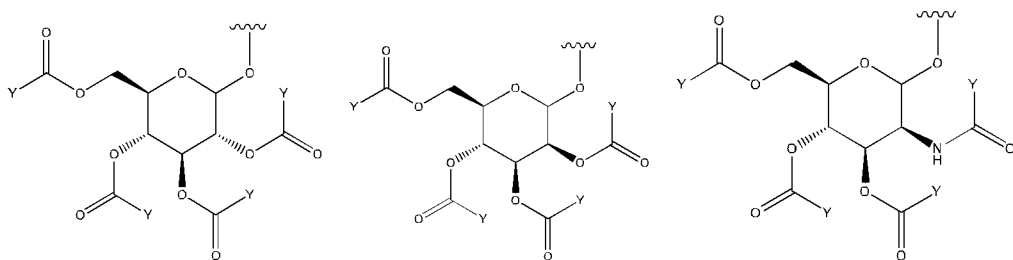
According to the above description, those skilled in the art that would easily understand that as compared with the well-known phosphoramidite solid phase synthesis method in the art, the siRNA conjugate as shown by Formula (308) in which the conjugation molecule is linked to any possible position of the nucleotide sequence can be obtained by using the above first functional group and an optional second functional group. For example, the conjugation molecule is linked to a terminal region of the nucleotide sequence, or to a terminal of the nucleotide sequence. Correspondingly, unless otherwise specified, in the following description regarding preparation of the conjugate and/or the conjugation molecule, when referring to the reactions such as “deprotection”, “coupling”, “capping”, “oxidation”, “sulfurization”, it will be understood that the reaction conditions and agents involved in the well-known phosphoramidite solid phase synthesis method in the art would also apply to these reactions. Exemplary reaction conditions and agents will be described in detail hereinafter.

25

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In some embodiments, each  $S_1$  is independently a  $M_1$ . In some embodiments, each  $S_1$  is independently a group formed by protecting at least one active hydroxyl group in  $M_1$  with a hydroxyl protecting group. In some embodiments, each  $S_1$  is independently a group formed by protecting all existing active hydroxyl groups in  $M_1$  with hydroxyl protecting groups. In some embodiments, any hydroxyl protecting group known to a skilled one  
 5 may be used to protect the active hydroxyl group in  $M_1$ . In some embodiments, the protected hydroxy is expressed as the Formula YCOO-, wherein each Y is independently selected from the group consisting of  $C_1$ - $C_{10}$  alkyl and  $C_6$ - $C_{10}$  aryl, which is optionally substituted with one or more substituents selected from the group consisting of halo and  $C_1$ - $C_6$  alkyl. In some embodiments, each Y is independently selected from the group consisting of methyl, trifluoromethyl, difluoromethyl, monofluoromethyl, trichloromethyl, dichloromethyl,  
 10 monochloromethyl, ethyl, n-propyl, isopropyl, phenyl, halophenyl, and  $C_1$ - $C_6$  alkylphenyl.

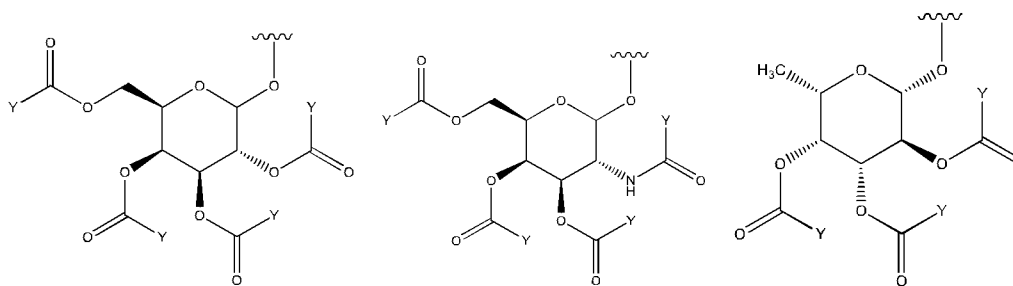
In some embodiments, each  $S_1$  is independently selected from the group consisting of Formulae (A46)- (A54):



(A46)

(A47)

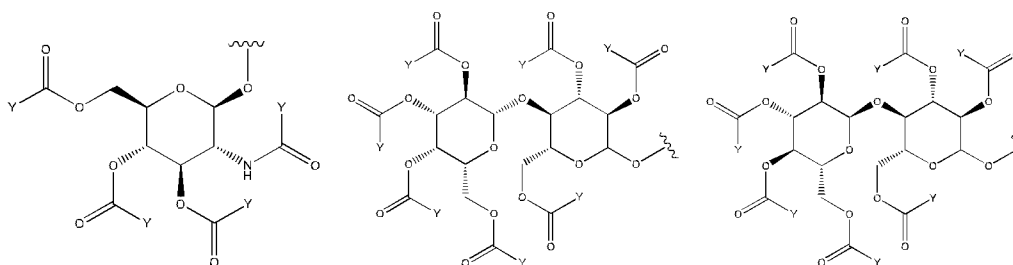
(A48)



(A49)

(A50)

(A51)



(A52)

(A53)

(A54)

20 In some embodiments,  $S_1$  is A49 or A50.

In some embodiments, each Y is independently one selected from methyl, trifluoromethyl, difluoromethyl, monofluoromethyl, trichloromethyl, dichloromethyl, monochloromethyl, ethyl, n-propyl, isopropyl, phenyl, halophenyl, and alkylphenyl. In some embodiments, Y is methyl.

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As mentioned above, the method for preparing the siRNA conjugate as shown by Formula (308) further comprises the following steps: synthesizing the other strand of the siRNA (for example, when a sense strand of the siRNA linked to a conjugation group is synthesized in the above step, the method further comprises synthesizing an antisense strand of the siRNA according to the solid phase synthesis method, *vice versa*),  
 5 isolating the sense strand and the antisense strand, and annealing. In particular, in the step of isolating, the solid phase support linked to the nucleotide sequence and/or the conjugation molecule is cleaved, and the necessary protecting group is removed (in this case, each S<sub>1</sub> group in the compound of Formula (321) is converted to the corresponding M<sub>1</sub> targeting group), to afford a sense strand (or an antisense strand) of the siRNA linked to a conjugation group and the corresponding antisense strand (or sense strand). The sense strand and the antisense  
 10 strand are annealed to form a double-strand RNA structure, thereby affording the siRNA conjugate as shown by Formula (308).

In some embodiments, the method for preparing the siRNA conjugate as shown by Formula (308) comprises the following steps: contacting the compound as shown by Formula (321) with the first nucleoside monomer  
 15 at 3' terminal of the sense strand or the antisense strand under coupling reaction condition in the presence of a coupling agent, thereby linking the compound as shown by Formula (321) to the first nucleotide in the sequence; sequentially linking nucleoside monomers in 3' to 5' direction to synthesize a sense or antisense strand of the siRNA according to the type and sequence of the nucleotides in the desired sense or antisense strand under the condition for phosphoramidite solid phase synthesis, wherein the compound of Formula (321) is a compound  
 20 in which R<sub>4</sub> comprises a first functional group and a second functional group, wherein the first functional group comprises a protected hydroxyl and the second functional group has a structure as shown by Formula (C1') or (C3'), and the compound of (321) is deprotected before being linked to the first nucleoside monomer; and the linking of each nucleoside monomer comprises a four-step reaction of deprotection, coupling, capping, and oxidation or sulfurization; thus obtaining a sense or antisense strand of the nucleic acid linked to a conjugation  
 25 group; sequentially linking nucleoside monomers in 3' to 5' direction to synthesize an antisense or sense strand of the nucleic acid according to the type and sequence of the nucleotides in the sense or antisense strand under the condition for phosphoramidite solid phase synthesis; wherein the linking of each nucleoside monomer includes a four-step reaction of deprotection, coupling, capping, and oxidation or sulfurization; removing the protecting group and cleaving the solid phase support; isolating and purifying the sense strand and the antisense  
 30 strand; and annealing.

In some embodiments, the method for preparing the siRNA conjugate as shown by Formula (308) comprises the following steps: according to the type and sequence of the nucleotides in the sense or antisense strand of the double-strand siRNA, sequentially linking nucleoside monomers in 3' to 5' direction to synthesize the  
 35 antisense and sense strand; wherein the linking of each nucleoside monomer includes a four-step reaction of deprotection, coupling, capping, and oxidation or sulfurization, to obtain the sense strand linked to the solid phase support and the antisense strand linked to the solid phase support; contacting the compound as shown by Formula (321) with the sense strand linked to the solid phase support or the antisense strand linked to the solid phase support under coupling reaction condition in the presence of a coupling agent, thereby linking the  
 40 compound as shown by Formula (321) to the sense strand or antisense strand; wherein the compound as shown by Formula (321) is a compound in which R<sub>4</sub> comprises a first functional group which is a phosphoramidite group; removing the protecting group and cleaving the solid phase support; respectively isolating and purifying the sense strand or the antisense strand of the siRNA; and annealing, wherein the sense or antisense strand of the siRNA is linked to a conjugation group.

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In some embodiments, the P atom in the Formula (A59) is linked to the 3' terminal of the sense strand of the siRNA, and the method for preparing the siRNA conjugate as shown by Formula (308) comprises:

- (1) removing the hydroxyl protecting group  $P_k$  in the compound of Formula (321), wherein the compound of Formula (321) is a compound in which  $R_4$  comprises a first functional group comprising a protected hydroxyl  $OR_k$ , and a second functional group having a structure as shown by Formulas (C1') or (C3'); contacting the deprotected product with a nucleoside monomer to afford a nucleoside monomer linked to a solid phase support via a conjugation molecule under coupling reaction condition in the presence of a coupling agent;
  - (2) starting from the nucleoside monomer linked to a solid phase support via the conjugation molecule, synthesizing a sense strand of the siRNA in 3' to 5' direction by a phosphoramidite solid phase synthesis method;
  - (3) synthesizing an antisense strand of the siRNA by a phosphoramidite solid phase synthesis method; and
  - (4) isolating the sense strand and the antisense strand of the siRNA and annealing the same to afford the siRNA conjugate as shown by Formula (308).
- Therein, in step (1), the method for removing the protecting group  $R_k$  in the above compound of Formula (321) comprises contacting the compound of Formula (321) with a deprotection agent under deprotection condition. The deprotection condition comprises a temperature of 0-50°C, and in some embodiments, 15-35°C, and a reaction time of 30-300 seconds, and in some embodiments, 50-150 seconds. The deprotection agent may be one or more selected from trifluoroacetic acid, trichloroacetic acid, dichloroacetic acid, and monochloroacetic acid, and in some embodiments, dichloroacetic acid. The molar ratio of the deprotection agent to the compound as shown by Formula (321) is 10:1 to 1000:1, and in some embodiments, 50:1 to 500:1.

The coupling reaction condition and the coupling agent may be any condition and agent suitable for the above coupling reaction. In some embodiments, the same condition and agent as those of the coupling reaction in the solid phase synthesis method can be used.

In some embodiments, the coupling reaction condition comprises a reaction temperature of 0-50°C, and in some embodiments, 15-35°C. The molar ratio of the compound of Formula (321) to the nucleoside monomer is 1:1 to 1:50, and in some embodiments, 1:2 to 1:5. The molar ratio of the compound of Formula (321) to the coupling agent may be 1:1 to 1:50, and in some embodiments, 1:3 to 1:10. The reaction time is 200-3,000 seconds, and in some embodiments, 500-1,500 seconds. The coupling agent is one or more selected from 1H-tetrazole, 5-ethylthio-1H-tetrazole and 5-benzylthio-1H-tetrazole, and in some embodiments, is 5-ethylthio-1H-tetrazole. The coupling reaction may be performed in an organic solvent. The organic solvent is one or more selected from anhydrous acetonitrile, anhydrous DMF and anhydrous dichloromethane, and in some embodiments, is anhydrous acetonitrile. With respect to the compound as shown by Formula (321), the amount of the organic solvent is 3-50 L/mol, and in some embodiments, 5-20 L/mol.

In step (2), starting from the nucleoside monomer linked to a solid phase support via a conjugation molecule prepared in the above steps, a sense strand SS of the siRNA conjugate is synthesized in 3' to 5' direction by the phosphoramidite solid phase synthesis method. In this case, the conjugation molecule is linked to 3' terminal of the resultant sense strand.

Other conditions for the solid phase synthesis in steps (2) and (3), including the deprotection condition for the nucleoside monomer, the type and amount of the deprotection agent, the coupling reaction condition, the type and amount of the coupling agent, the capping reaction condition, the type and amount of the capping agent, the oxidation reaction condition, the type and amount of the oxidation agent, the sulfuration reaction condition,

and the type and amount of the sulfurization agent, adopt various conventional agents, amounts, and conditions in the art.

5 In some embodiments, for example, the solid phase synthesis in steps (2) and (3) can use the following conditions:

The deprotection condition for the nucleoside monomer comprises a reaction temperature of 0-50°C, and in some embodiments, 15-35°C, and a reaction time of 30-300 seconds, and in some embodiments, 50-150 seconds. The deprotection agent may be one or more selected from trifluoroacetic acid, trichloroacetic acid, 10 dichloroacetic acid, and monochloroacetic acid, and in some embodiments, is dichloroacetic acid. The molar ratio of the deprotection agent to the protecting group 4,4'-dimethoxytrityl on the solid phase support is 2:1 to 100:1, and in some embodiments, 3:1 to 50:1.

15 The coupling reaction condition comprises a reaction temperature of 0-50°C, and in some embodiments, 15-35°C. The molar ratio of the nucleic acid sequence linked to the solid phase support to the nucleoside monomer is 1:1 to 1:50, and in some embodiments, 1:5 to 1:15. The molar ratio of the nucleic acid sequence linked to the solid phase support to the coupling agent is 1:1 to 1:100, and in some embodiments, 1:50 to 1:80. The selection of the reaction time and the coupling agent is the same as above.

20 The capping reaction condition comprises a reaction temperature of 0-50°C, and in some embodiments, 15-35°C, and a reaction time of 5-500 seconds, and in some embodiments, 10-100 seconds. The selection of the capping agent is the same as above. The molar ratio of the total amount of the capping agent to the nucleic acid sequence linked to the solid phase support is 1:100 to 100:1, and in some embodiments, is 1:10 to 10:1. In the case where the capping agent uses equimolar acetic anhydride and N-methylimidazole, the molar ratio of acetic 25 anhydride, N-methylimidazole, and the nucleic acid sequence linked to the solid phase support may be 1:1:10 - 10:10:1, and in some embodiments, is 1:1:2 - 2:2:1.

30 The oxidation reaction condition comprises a reaction temperature of 0-50°C, and in some embodiments, 15-35°C, and a reaction time of 1-100 seconds, and in some embodiments, 5-50 seconds. In some embodiments, the oxidation agent is iodine (in some embodiments provided as iodine water). The molar ratio of the oxidation agent to the nucleic acid sequence linked to the solid phase support in the coupling step may be 1:1 to 100:1, and in some embodiments, is 5:1 to 50:1. In some embodiments, the oxidation reaction is performed in a mixed solvent in which the ratio of tetrahydrofuran: water: pyridine is 3:1:1-1:1:3. The sulfurization reaction condition 35 comprises a reaction temperature of 0-50°C, and in some embodiments, 15-35°C, and a reaction time of 50-2,000 seconds, and in some embodiments, 100-1,000 seconds. In some embodiments, the sulfurization agent is xanthane hydride. The molar ratio of the sulfurization agent to the nucleic acid sequence linked to the solid phase support in the coupling step is 10:1 to 1,000:1, and in some embodiments, is 10:1 to 500:1. In some embodiments, the sulfurization reaction is performed in a mixed solvent in which the ratio of acetonitrile: pyridine is 1:3-3:1.

40 The method further comprises isolating the sense strand and the antisense strand of the siRNA after linking all nucleoside monomers and before the annealing. Methods for isolation are well-known to those skilled in the art and generally comprise cleaving the synthesized nucleotide sequence from the solid phase support, removing the protecting groups on the bases, phosphate groups and ligands, purifying, and desalting.

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- The conventional cleavage and deprotection methods in the synthesis of siRNAs can be used to cleave the synthesized nucleotide sequence from the solid phase support, and remove the protecting groups on the bases, phosphate groups and ligands. For example, the resultant nucleotide sequence linked to the solid phase support is contacted with concentrated aqueous ammonia; during deprotection, the protecting group YCOO- in groups A46-A54 is converted to a hydroxyl group, and thus the S<sub>1</sub> groups are converted to corresponding M<sub>1</sub> groups, providing the siRNA conjugate as shown by Formula (308); wherein the concentrated aqueous ammonia may be aqueous ammonia of a concentration of 25-30 wt%. With respect to the target siRNA sequence, the amount of the concentrated aqueous ammonia may be 0.2 ml/μmol-0.8 ml/μmol.
- When there is at least one 2'-TBDMS protection on the synthesized nucleotide sequence, the method further comprises contacting the nucleotide sequence removed from the solid phase support with triethylamine trihydrofluoride to remove the 2'-TBDMS protection. Here, the corresponding nucleotide in the resultant target siRNA sequence has free 2'-hydroxy. With respect to the target siRNA sequence, the amount of pure triethylamine trihydrofluoride is 0.4 ml/μmol-1.0 ml/μmol. As such, the siRNA conjugate as shown by Formula (308) can be obtained.

Methods for purification and desalination are well-known to those skilled in the art. For example, nucleic acid purification may be performed using a preparative ion chromatography purification column with a gradient elution of NaBr or NaCl; after collection and combination of the product, the desalination may be performed using a reverse phase chromatography purification column.

In the resultant siRNA conjugate as shown by Formula (308), the non-bridging oxygen or sulfur atom in the phosphodiester bond or phosphorothioate diester bond between the nucleotides substantially binds to sodium ion, and the siRNA conjugate as shown by Formula (308) is substantially present in the form of a sodium salt. The well-known ion-exchange methods may be used, in which the sodium ion may be replaced with hydrogen ion and/or other cations, thereby providing other forms of siRNA conjugates as shown by Formula (308). The cations are as described above.

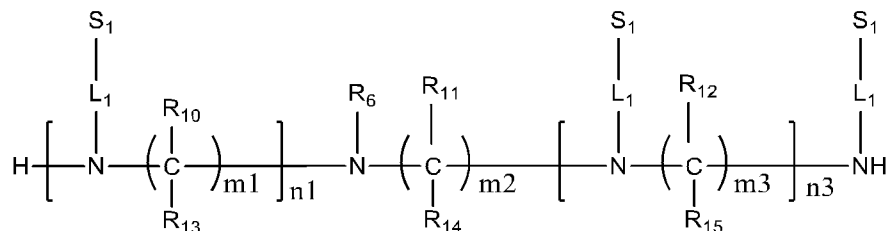
During synthesis, the purity and molecular weight of the nucleic acid sequence may be determined at any time, in order to better control the synthesis quality. Such determination methods are well-known to those skilled in the art. For example, the purity of the nucleic acid may be determined by ion exchange chromatography, and the molecular weight may be determined by liquid chromatography-mass spectrometry (LC-MS).

Methods for annealing are also well-known to those skilled in the art. For example, the synthesized sense strand (SS strand) and the antisense strand (AS strand) may be simply mixed in water for injection at an equimolar ratio, heated to 70-95°C, and then cooled at room temperature to form a double-stranded structure via hydrogen bond. Hence, the siRNA conjugate as shown by Formula (308) can be obtained.

After having obtained the conjugate, in some embodiments, the synthesized siRNA conjugate as shown by Formula (308) can also be characterized by the means such as molecular weight detection using the methods such as liquid chromatography-mass spectrometry, to confirm that the synthesized siRNA conjugate is the designed siRNA conjugate as shown by Formula (308) of interest, and the sequence of the synthesized siRNA is the sequence of the desired siRNA sequence, for example, one of the sequences listed in Tables 1a-1g.

The compound as shown by Formula (321) may be obtained by the following preparation method, comprising: contacting a compound as shown by Formula (313) with a cyclic anhydride in an organic solvent under

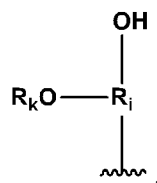
esterification reaction condition in the presence of a base and an esterification catalyst; isolating the compound as shown by Formula (321) by ion exchange:



Formula (313)

wherein the definitions and options of  $n_1$ ,  $n_3$ ,  $m_1$ ,  $m_2$ ,  $m_3$ ,  $R_{10}$ ,  $R_{11}$ ,  $R_{12}$ ,  $R_{13}$ ,  $R_{14}$ ,  $R_{15}$ ,  $L_1$ , and  $S_1$  are respectively as described above;

$R_6$  is a group for providing  $R_4$  of Formula (321); in some embodiments,  $R_6$  has a structure as shown by Formula (A61):



(A61)

wherein  $R_i$  is any group capable of linking to the N atom on the nitrogenous backbone, linking to  $R_k\text{O}$  and linking to a free hydroxy group;  $R_k$  is a hydroxy protecting group. In this case, a compound as shown by Formula (321) is obtained, wherein  $R_4$  comprises a first functional group which comprises a hydroxy protecting group and a second functional group which comprises a structure as shown by Formula (C1) or (C2).

The esterification reaction condition includes a reaction temperature of 0-100°C and a reaction time of 8-48 hours. In some embodiments, the esterification reaction condition comprises a reaction temperature of 10-40°C and a reaction time of 20-30 hours.

In some embodiments, the organic solvent comprises one or more of an epoxy solvent, an ether solvent, an haloalkane solvent, dimethyl sulfoxide, N,N-dimethylformamide, and N,N-diisopropylethylamine. In some embodiments, the epoxy solvent is dioxane and/or tetrahydrofuran, the ether solvent is diethyl ether and/or methyl tertbutyl ether, and the haloalkane solvent is one or more of dichloromethane, trichloromethane and 1,2-dichloroethane. In some embodiments, the organic solvent is dichloromethane. With respect to the compound as shown by Formula (313), the amount of the organic solvent is 3-50 L/mol, and in some embodiments, 5-20 L/mol.

In some embodiments, the cyclic anhydride is one of succinic anhydride, glutaric anhydride, adipic anhydride or pimelic anhydride, and in some embodiments, the cyclic anhydride is succinic anhydride. The molar ratio of the cyclic anhydride to the compound as shown by Formula (313) is 1:1 to 10:1, and in some embodiments, 2:1 to 5:1.

The esterification catalyst may be any catalyst capable of catalyzing esterification reaction, such as 4-dimethylaminopyridine. The molar ratio of the catalyst to the compound as shown by Formula (313) is 1:1 to 10:1, and in some embodiments, is 2:1 to 5:1.

- 5 In some embodiments, the base may be any inorganic base, organic base or combination thereof. Considering solubility and product stability, the base may be, for example, tertiary amine. In some embodiments, the tertiary amine is triethylamine or N,N-diisopropylethylamine. The molar ratio of the tertiary amine to the compound as shown by Formula (313) is 1:1 to 20:1, and in some embodiments, 3:1 to 10:1.
- 10 The ion exchange serves the function of converting the compound as shown by Formula (321) into a desired form of carboxylic acid or carboxylic salt and the methods of ion exchange are well-known to those skilled in the art. The conjugation molecule having a  $M^+$  cation may be obtained by using suitable ion exchange solution and ion exchange condition, which are omitted herein. In some embodiments, the ion exchange reaction is performed using a triethylamine phosphate solution, and the concentration of the triethylamine phosphate
- 15 solution is 0.2-0.8 M. In some embodiments, the concentration of the triethylamine phosphate solution is 0.4-0.6 M, and with respect to the compound as shown by Formula (313), the amount of the triethylamine phosphate solution is 3-6 L/mol, and in further embodiments, 4-5 L/mol.

- The compound of Formula (321) may be isolated from the reaction mixture using any suitable isolation methods.
- 20 In some embodiments, the compound of Formula (321) may be isolated by removal of solvent via evaporation followed by chromatography. For example, the following two chromatographic conditions can be used for isolation: (1) normal phase purification of silica gel: 200-300 mesh silica gel filler, gradient elution of 1 wt% triethylamine in dichloromethane: methanol = 100:18 - 100:20; or (2) reverse phase purification: C18 and C8 reverse phase filler, gradient elution of methanol: acetonitrile = 0.1: 1 - 1:0.1. In some embodiments, the solvent
- 25 may be directly removed to obtain a crude product of the compound of Formula (321), which may be directly used in subsequent reactions.

- In some embodiments, the method for preparing the compound of Formula (321) further comprises: further contacting the product obtained by the above ion exchanging reaction with a solid phase support with amino
- 30 or hydroxy groups in an organic solvent under condensation reaction condition in the presence of a condensation agent, a condensation catalyst and a tertiary amine. In this case, a compound as shown by Formula (321) is obtained, wherein  $R_4$  comprises a first functional group which comprises a hydroxy protecting group and a second functional group which comprises a structure as shown by Formula (C1').

- 35 The solid phase support is one of the supports used in solid phase synthesis of siRNA, some of which are well-known to those skilled in the art. For example, the solid phase support may be selected from the solid phase supports containing active hydroxy or amino functional group(s), and in some embodiments, is an amino or hydroxy resin. In some embodiments, the amino or hydroxy resin has the following parameters: particle size of 100-400 mesh, and surface amino or hydroxy loading of 0.2 - 0.5 mmol/g. The ratio of the compound as
- 40 shown by Formula (321) to the solid phase support is 10 - 400  $\mu$ mol compound/g solid phase support. In some embodiments, the ratio of the compound of Formula (321) to the solid phase support is 50  $\mu$ mol/g to 200  $\mu$ mol/g.

- The organic solvent may be any suitable solvent or mixed solvent known to those skilled in the art. In some
- 45 embodiments, the organic solvent is one or more of acetonitrile, an epoxy solvent, an ether solvent, an haloalkane solvent, dimethyl sulfoxide, N,N-dimethylformamide, and N,N-diisopropylethylamine. In some embodiments, the epoxy solvent is dioxane and/or tetrahydrofuran; the ether solvent is diethyl ether and/or

methyl tertbutyl ether; the haloalkane solvent is one or more of dichloromethane, trichloromethane and 1,2-dichloroethane. **In some embodiments**, the organic solvent is acetonitrile. With respect to the compound of Formula (321), the amount of the organic solvent is 20-200 L/mol, and **in some embodiments**, 50-100 L/mol.

5 In some embodiments, the condensation agent may be benzotriazol-1-yl-oxytripyrrolidino phosphonium hexafluorophosphate (PyBop), 3-diethoxyphosphoryl-1,2,3-benzotriazin-4(3H)-one (DEPBT) and/or O-benzotriazol-tetramethyluronium hexafluorophosphate. **In some embodiments**, the condensation agent is O-benzotriazol-tetramethyluronium hexafluorophosphate. The molar ratio of the condensation agent to the compound as shown by Formula (321) is 1:1 to 20:1, and **in other embodiments**, 1:1 to 5:1.

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In some embodiments, the tertiary amine is triethylamine and/or N,N-diisopropylethylamine, and **in some embodiments**, N,N-diisopropylethylamine. The molar ratio of the tertiary amine to the compound as shown by Formula (321) is 1:1 to 20:1, and **in some embodiments**, 1:1 to 5:1.

15 In some embodiments, the method for preparing the compound of Formula (321) further comprises: contacting the resultant condensation product with a capping agent and an acylation catalyst in an organic solvent under capping reaction condition, and isolating the compound of Formula (321). The capping reaction is used to remove any active functional group that does not completely react, so as to avoid producing unnecessary by-products in subsequent reactions. The capping reaction condition comprises a reaction temperature of 0-50°C, and **in some embodiments**, 15-35°C, and a reaction time of 1-10 hours, and **in some embodiments**, 3-6 hours. The capping agent may be the capping agent used in solid phase synthesis of siRNA, which are well-known to those skilled in the art.

25 In some embodiments, the capping agent is composed of a capping agent 1 (cap1) and a capping agent 2 (cap2). The cap1 is N-methylimidazole, and **in some embodiments**, provided as a mixed solution of N-methylimidazole in pyridine/acetonitrile, wherein the volume ratio of pyridine to acetonitrile is 1:10 to 1:1, and **in some embodiments**, 1:3 to 1:1. **In some embodiments**, the ratio of the total volume of pyridine and acetonitrile to the volume of N-methylimidazole is 1:1 to 10:1, and **in some embodiments**, 3:1 to 7:1. The cap2 is acetic anhydride, and **in some embodiments**, provided as a solution of acetic anhydride in acetonitrile, wherein the volume ratio of acetic anhydride to acetonitrile is 1:1 to 1:10, and **in further embodiments**, 1:2 to 1:6.

35 In some embodiments, the ratio of the volume of the mixed solution of N-methylimidazole in pyridine/acetonitrile to the weight of the compound of Formula (321) is 5 ml/g-50 ml/g, and **in some embodiments**, 15 ml/g-30 ml/g. The ratio of the volume of the solution of acetic anhydride in acetonitrile to the weight of the compound of Formula (321) is 0.5 ml/g-10 ml/g, and **in some embodiments**, 1 ml/g-5 ml/g.

**In some embodiments**, the capping agent comprises equimolar acetic anhydride and N-methylimidazole. The organic solvent is one or more of acetonitrile, an epoxy solvent, an ether solvent, an haloalkane solvent, dimethyl sulfoxide, N,N-dimethylformamide, and N,N-diisopropylethylamine. **In some embodiments**, the organic solvent is acetonitrile. With respect to the compound of Formula (321), the amount of the organic solvent is 10-50 L/mol, and **in some embodiments**, 5-30 L/mol.

45 **In some embodiments**, the acylation catalyst may be selected from any catalyst that may be used for esterification condensation or amidation condensation, such as alkaline heterocyclic compounds. **In some embodiments**, the acylation catalyst is 4-dimethylaminopyridine. The weight ratio of the catalyst to the compound as shown by Formula (321) may be 0.001:1 to 1:1, and **in some embodiments**, 0.01:1 to 0.1:1.

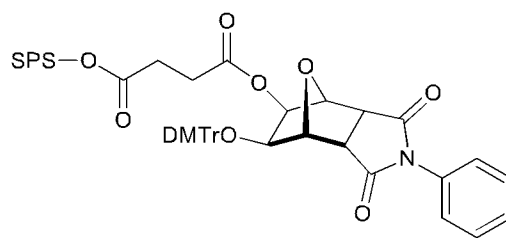
In some embodiments, the compound of Formula (321) may be isolated from the reaction mixture by any suitable separation methods. In some embodiments, the compound of Formula (321) may be obtained by thoroughly washing with an organic solvent and filtering to remove unreacted reactants, excess capping agent and other impurities, wherein the organic solvent is selected from acetonitrile, dichloromethane and methanol. In some embodiments, the organic solvent is acetonitrile.

In some embodiments, the preparation method of the conjugation molecule as shown by Formula (321) comprises contacting a compound as shown by Formula (313) with a phosphorodiamidite in an organic solvent under coupling reaction condition in the presence of a coupling agent, and isolating the compound as shown by Formula (321). In this case, a compound as shown by Formula (321) is obtained, wherein  $R_4$  comprises a first functional group comprising a hydroxy protecting group and a second functional group comprising a structure as shown by Formula (C3).

In some embodiments, the coupling reaction condition comprises: a reaction temperature of 0-50°C, such as 15-35°C; the molar ratio of the compound of Formula (313) to the phosphorodiamidite of 1:1 to 1:50, such as 1:5 to 1:15; the molar ratio of the compound of Formula (313) to the coupling agent of 1:1 to 1:100, such as 1:50 to 1:80; and a reaction time of 200-3,000 seconds, such as 500-1,500 seconds. The phosphorodiamidite may be, for example, bis(diisopropylamino)(2-cyanoethoxy)phosphine, which may be commercially available or synthesized according to the methods well-known in the art. The coupling agent is one or more selected from 1H-tetrazole, 5-ethylthio-1H-tetrazole and 5-benzylthio-1H-tetrazole, such as 5-ethylthio-1H-tetrazole. The coupling reaction may be performed in an organic solvent. The organic solvent is one or more selected from anhydrous acetonitrile, anhydrous DMF and anhydrous dichloromethane, such as anhydrous acetonitrile. With respect to the compound of Formula (313), the amount of the organic solvent is 3-50 L/mol, such as 5-20 L/mol. By coupling reaction, the hydroxy group in the compound of Formula (313) reacts with the phosphorodiamidite to form a phosphoramidite group. In some embodiments, the solvent may be directly removed to afford a crude product of the compound of Formula (321), which may be directly used in subsequent reactions.

In some embodiments, the preparation method of the compound of Formula (321) further comprises the following steps: further contacting the isolated product with a solid phase support with hydroxy groups in an organic solvent under coupling reaction condition in the presence of a coupling agent, followed by capping, oxidation, and isolation, to afford the compound of Formula (321), wherein  $R_4$  comprises a first functional group comprising a hydroxy protecting group and a second functional group comprising a structure as shown by Formula (C3').

In some embodiments, the solid phase support is a solid support well-known in the art for solid phase synthesis of nucleic acid, such as, a deprotected commercially available universal solid phase support (NittoPhase®HL UnyLinker™ 300 Oligonucleotide Synthesis Support, Kinovate Life Sciences, as shown by Formula (B80)):



(B80).

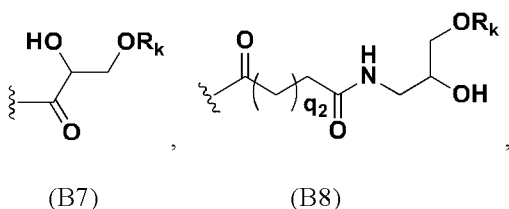
A deprotection reaction is well-known to those skilled in the art. In some embodiments, the deprotection condition comprises a temperature of 0-50°C, such as 15-35°C, and a reaction time of 30-300 seconds, such as 50-150 seconds. The deprotection agent may be one or more selected from of trifluoroacetic acid, trichloroacetic acid, dichloroacetic acid, and monochloroacetic acid. In some embodiments, the deprotection agent is dichloroacetic acid. The molar ratio of the deprotection agent to the protecting group -DMTr (4,4' - dimethoxytrityl) on the solid phase support is 2:1 to 100:1, such as 3:1 to 50:1. Through such deprotection, reactive free hydroxy groups are obtained on the surface of the solid phase support, for facilitating the subsequent coupling reaction.

The coupling reaction condition and the coupling agent may be selected as above. Through the coupling reaction, the free hydroxy groups formed in the deprotection react with the phosphoramidite groups, so as to form a phosphite ester linkage.

In some embodiments, the capping reaction condition comprises a temperature of 0-50°C, such as 15-35°C, and a reaction time of 5-500 seconds, such as 10-100 seconds. The capping reaction is carried out in the presence of a capping agent. The selection and amount of the capping agent are as described above.

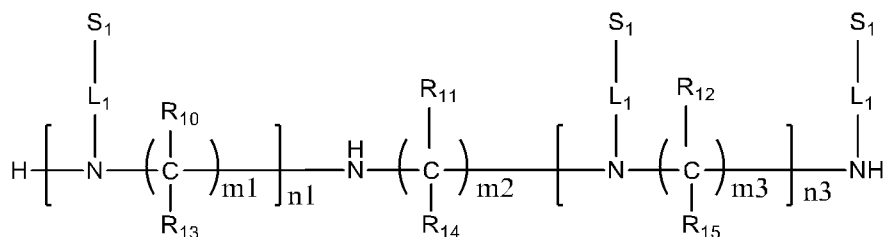
The oxidation reaction condition comprises a temperature of 0-50°C, such as 15-35°C, and a reaction time of 1-100 seconds, such as 5-50 seconds. The oxidation agent may be, for example, iodine (in some embodiments, provided as iodine water). The molar ratio of the oxidation agent to the nucleic acid sequence linked to the solid phase support is 1:1 to 100:1, such as, may be 5:1 to 50:1. In some embodiments, the oxidation reaction is performed in a mixed solvent in which the ratio of tetrahydrofuran: water: pyridine = 3:1:1 - 1:1:3.

In some embodiments,  $R_k$  is one of the groups of Formula (B7) or (B8):

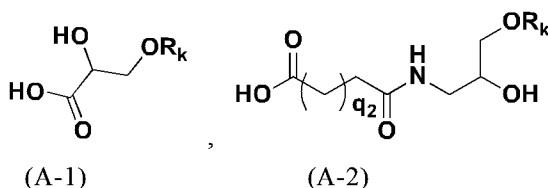


wherein the definitions of  $q_2$  and  $R_k$  are as described above.

In this case, the compound as shown by Formula (313) may be obtained by the following preparation method, comprising: contacting the compound as shown by Formula (314) with a compound as shown by Formula (A-1) or (A-2) in an organic solvent under amidation reaction condition in the presence of a condensation agent for amidation reaction and an tertiary amine, followed by isolation:



Formula (314)



5

wherein the definitions and options of  $n1$ ,  $n3$ ,  $m1$ ,  $m2$ ,  $m3$ ,  $R_{10}$ ,  $R_{11}$ ,  $R_{12}$ ,  $R_{13}$ ,  $R_{14}$ ,  $R_{15}$ ,  $L_1$ ,  $S_1$ ,  $q_2$ , and  $R_k$  are respectively as described above.

10 The amidation reaction condition comprises a reaction temperature of 0-100°C and a reaction time of 1-48 hours. In some embodiments, the amidation reaction condition comprises a reaction temperature of 10-40°C and a reaction time of 2-16 hours.

15 In some embodiments, the organic solvent is one or more of an alcohol solvent, an epoxy solvent, an ether solvent, an haloalkane solvent, dimethyl sulfoxide, N,N-dimethylformamide, and N,N-diisopropylethylamine. In some embodiments, the alcohol solvent is one or more of methanol, ethanol and propanol, and in some embodiments, ethanol. In some embodiments, the epoxy solvent is dioxane and/or tetrahydrofuran. In some embodiments, the ether solvent is diethyl ether and/or methyl tert-butyl ether. In some embodiments, the haloalkane solvent is one or more of dichloromethane, trichloromethane and 1,2-dichloroethane. In some  
20 **embodiments**, the organic solvent is dichloromethane. With respect to the compound of Formula (314), the amount of the organic solvent is 3-50 L/mol, and in some **embodiments**, 3-20 L/mol.

In some embodiments, the condensation agent for amidation reaction is benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, 3-diethoxyphosphoryl-1,2,3-benzotriazin-4(3H)-one, 4-  
25 (4,6-dimethoxytriazin-2-yl)-4-methylmorpholine hydrochloride, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), or O-benzotriazol-tetramethyluronium hexafluorophosphate, and in further **embodiments**, 3-diethoxyphosphoryl-1,2,3-benzotriazin-4(3H)-one. The molar ratio of the condensation agent for amidation reaction to the compound as shown by Formula (314) is 1:1 to 10:1, and in some **embodiments**, 2.5:1 to 5:1.

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In some embodiments, the tertiary amine is triethylamine or N,N-diisopropylethylamine, and in some **embodiments**, N,N-diisopropylethylamine. The molar ratio of the tertiary amine to the compound as shown by Formula (314) is 3:1 to 20:1, and in some **embodiments**, 5:1 to 10:1.

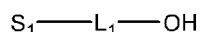
35 The compounds of Formula (A-1) and (A-2) may be prepared by any suitable means. For example, when  $R_k$  is a DMTr group, the compound of Formula (A-1) may be prepared by reacting calcium glycerate with DMTrCl.

Similarly, the compound of Formula (A-2) may be prepared by firstly contacting 3-amino-1,2-propanediol with a cyclic anhydride and then reacting with DMTrCl, wherein the cyclic anhydride may have 4-13 carbon atoms, and in some embodiments, 4-8 carbon atoms. Those skilled in the art would easily understand that the selections of different cyclic anhydrides correspond to different values for  $q_2$  in the compound of Formula (A-2). For example, when the cyclic anhydride is succinic anhydride,  $q_2=1$ ; when the cyclic anhydride is glutaric anhydride,  $q_2=2$ , and so on.

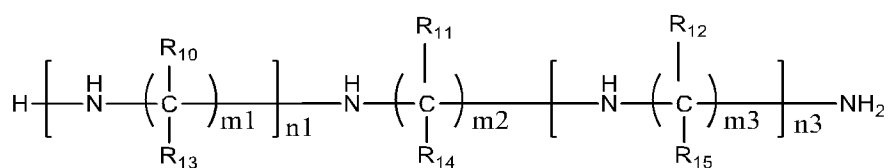
In some variations, the compound of Formula (313) can also be prepared by sequentially reacting the compound as shown by Formula (314) with the cyclic anhydride, 3-amino-1,2-propanediol and DMTrCl. Those skilled in the art would easily understand that these variations would not affect the structure and function of the compound of Formula (313), and these variations are readily realized by those skilled in the art on the basis of the above methods.

Similarly, the compound of Formula (313) may be isolated from the reaction mixture by any suitable isolation methods. In some embodiments, the compound of Formula (313) may be isolated by removal of solvent via evaporation followed by chromatography. For example, the following chromatographic conditions may be used for isolation: (1) normal phase purification of silica gel: 200-300 mesh silica gel filler, with gradient elution of petroleum ether: ethyl acetate: dichloromethane: N,N-dimethylformamide = 1:1:1:0.5 - 1:1:1:0.6; and (2) reverse phase purification: C18 and C8 reverse phase fillers, with gradient elution of methanol: acetonitrile = 0.1:1 - 1:0.1. In some embodiments, the solvent may be directly removed to afford a crude product of the compound of Formula (313), which may be directly used in subsequent reactions.

In some embodiments, the compound as shown by Formula (314) may be obtained by the following preparation method, comprising: contacting the compound as shown by Formula (320) with the compound as shown by Formula (316) in an organic solvent under condensation reaction condition in the presence of a condensation agent for amidation reaction and a tertiary amine, followed by isolation:



Formula (316)



Formula (320)

wherein the definitions and options of  $n1$ ,  $n3$ ,  $m1$ ,  $m2$ ,  $m3$ ,  $R_{10}$ ,  $R_{11}$ ,  $R_{12}$ ,  $R_{13}$ ,  $R_{14}$ , and  $R_{15}$  are respectively as described above.

The compound of Formula (316) can be, such as, those disclosed in J. Am. Chem. Soc. 2014, 136, 16958-16961. Alternatively, the compounds of Formula (316) may be prepared by those skilled in the art via various methods. For example, some compounds of Formula (316) may be prepared according to the method disclosed in Example 1 of the US patent US8106022B2, which is incorporated herein by reference in its entirety.

In some embodiments, the condensation reaction condition comprises a reaction temperature of 0-100°C and a reaction time of 0.1-24 hours, and in some embodiments, a reaction temperature of 10-40°C and a reaction time of 0.5-16 hours.

- 5 Considering the structure of the desired product compound of Formula (314), the molar ratio of the compound as shown by Formula (316) to the compound as shown by Formula (320) should be determined based on the sum of  $n_1$  and  $n_3$  in Formula (320). In some embodiments, for example, when  $n_1+n_3=3$ , to ensure complete reaction without any excess, the molar ratio of the compound as shown by Formula (316) to the compound as shown by Formula (320) may be 3:1 to 3.5:1, and in some embodiments, 3.01:1 to 3.15:1.

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In some embodiments, the organic solvent is one or more of acetonitrile, an epoxy solvent, an ether solvent, an haloalkane solvent, dimethyl sulfoxide, N,N-dimethylformamide, and N,N-diisopropylethylamine. In some embodiments, the epoxy solvent is dioxane and/or tetrahydrofuran. In some embodiments, the ether solvent is diethyl ether and/or methyl tert-butyl ether. In some embodiments, the haloalkane solvent is one or more of dichloromethane, trichloromethane and 1,2-dichloroethane. In some embodiments, the organic solvent is dichloromethane. With respect to the compound of Formula (320), the amount of the organic solvent may be 3-50 L/mol, and in some embodiments, 5-20 L/mol.

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- 20 In some embodiments, the condensing agent for amidation reaction is benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, 3-diethoxyphosphoryloxy-1,2,3-benzotriazin-4(3H)-one (DEPBT), O-benzotriazol-tetramethyluronium hexafluorophosphate, 4-(4,6-dimethoxytriazin-2-yl)-4-methylmorpholine hydrochloride, or 1-hydroxybenzotriazole, and in further embodiments, is a mixture of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate and 1-hydroxybenzotriazole, wherein benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate and 1-hydroxybenzotriazole are used in equimolar amounts. The molar ratio of the total condensing agent for amidation reaction to the compound as shown by Formula (316) may be 1:1 to 3:1, and in some embodiments, 1.05:1 to 1.5:1.

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- The tertiary amine may be N-methylmorpholine, triethylamine or N,N-diisopropylethylamine, and in some embodiments, N-methylmorpholine. The molar ratio of the tertiary amine to the compound as shown by Formula (316) may be 2:1 to 10:1, and in some embodiments, 2:1 to 5:1.

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- Similarly, the compound as shown by Formula (314) may be isolated from the reaction mixture by any suitable isolation method. In some embodiments, the compound as shown by Formula (314) may be isolated by removal of solvent via evaporation followed by chromatography, for example, using the following two chromatographic conditions for isolation: (1) normal phase purification of silica gel: 200-300 mesh silica gel filler, with gradient elution of dichloromethane: methanol = 100:5 - 100:7; and (2) reverse phase purification: C<sub>18</sub> and C<sub>8</sub> reverse phase fillers, with gradient elution of methanol: acetonitrile = 0.1:1 - 1:0.1. In some embodiments, the solvent may be directly removed to afford a crude product of the compound of Formula (314), which may be directly used in subsequent reactions.

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The compound of Formula (320) may be commercially available, or prepared by those skilled in the art via known methods. For example, in the case where  $m_1=m_2=m_3=3$ ,  $n_1=1$ ,  $n_3=2$ , and each of R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub>, R<sub>14</sub>, and R<sub>15</sub> is H, the compound of Formula (320) may be commercially available from Alfa Aesar Inc.

- 45 The siRNA conjugate of the present disclosure may also be used in combination with other pharmaceutically acceptable excipients, which may be one or more of various conventional formulations or compounds in the

art. For details, please refer to the above description of the pharmaceutical compositions of the present disclosure.

**Use of the siRNA, the pharmaceutical composition and the conjugate of the present disclosure**

5 In some embodiments, the present disclosure provides the use of the siRNA and/or the pharmaceutical composition and/or the siRNA conjugate of the present disclosure in the manufacture of a medicament for treating and/or preventing diseases or physiological conditions caused by abnormal expression of PCSK9 gene.

10 In some embodiments, the present disclosure provides a method for preventing and/or treating diseases or physiological conditions caused by abnormal expression of PCSK9 gene, comprising administering an effective amount of the siRNA and/or the pharmaceutical composition and/or the siRNA conjugate of the present disclosure to a subject in need thereof.

15 The purpose of preventing and/or treating diseases or physiological conditions caused by abnormal expression of PCSK9 gene may be achieved through the mechanism of RNA interference by administering the siRNA active ingredient of the present disclosure to a subject in need thereof. Therefore, the siRNA and/or the pharmaceutical composition and/or the siRNA conjugate of the present disclosure may be used for preventing and/or treating diseases or physiological conditions caused by abnormal expression of PCSK9 gene, or for preparing a medicament for preventing and/or treating diseases or physiological conditions caused by abnormal expression of PCSK9 gene.

20 In some embodiments, the diseases or physiological conditions caused by abnormal expression of PCSK9 gene refer to hypercholesterolemia, and resulting cardiovascular diseases including atherosclerosis, coronary heart disease and hypertension.

25 As used herein, the term “administration/administer” refers to the delivery of the siRNA and/or the pharmaceutical composition and/or the siRNA conjugate of the present disclosure into a subject’s body by a method or a route that at least partly locates the siRNA and/or the pharmaceutical composition and/or the siRNA conjugate of the present disclosure at a desired site to produce a desired effect. Suitable administration routes for the methods of the present disclosure include topical administration and systemic administration. In general, topical administration results in the delivery of more siRNA conjugates to a particular site as compared with the systemic circulation of the subject; whereas systemic administration results in the delivery of the siRNA and/or the pharmaceutical composition and/or the siRNA conjugate of the present disclosure to the basic systemic circulation of the subject. Considering that the present disclosure is intended to provide a means for preventing and/or treating hypercholesterolemia, in some embodiments, an administration mode capable of delivering a medicament to the liver is used.

40 The administration to a subject may be achieved by any suitable routes known in the art, including but not limited to, oral and parenteral routes, such as intravenous administration, intramuscular administration, subcutaneous administration, transdermal administration, intratracheal administration (aerosol), pulmonary administration, nasal administration, rectal administration, and topical administration (including buccal administration and sublingual administration). The administration frequency may be once or more times daily, weekly, biweekly, triweekly, monthly, bimonthly, quarterly, semiannually, or yearly.

45 The dose of the siRNA or the pharmaceutical composition or the siRNA conjugate of the present disclosure may be a conventional dose in the art, which may be determined according to various parameters, especially

age, weight and gender of a subject. Toxicity and efficacy may be determined in cell cultures or experimental animals by standard pharmaceutical procedures, for example, by determining LD<sub>50</sub> (the lethal dose that causes 50% population death), ED<sub>50</sub> (the dose that can cause 50% of the maximum response intensity in a graded response, and that can cause 50% of the experimental subjects to have a positive response in a qualitative response), or IC<sub>50</sub> (the concentration of an inhibitor/a drug where the graded response is inhibited by half). The dose range for human may be derived based on data obtained from cell culture analysis and animal studies.

When the siRNA, the pharmaceutical composition and/or the siRNA conjugate of the present disclosure is administered, for example, to male or female, 3-5 year old cynomolgus monkeys weighing 2 to 6 kg, (i) for the siRNA conjugate, the amount of the siRNA thereof may be 0.001 to 100 mg/kg body weight, in some embodiments 0.01 to 50 mg/kg body weight, in some embodiments 0.05 to 20 mg/kg body weight, in other embodiments 0.1 to 15 mg/kg body weight, and in other embodiments 0.1 to 10 mg/kg body weight; (ii) for a pharmaceutical composition formed from the siRNA and the pharmaceutically acceptable carrier, the amount of the siRNA thereof may be 0.001 to 50 mg/kg body weight, in some embodiments 0.01 to 10 mg/kg body weight, in some embodiments 0.05 to 5 mg/kg body weight, and in some embodiments 0.1 to 3 mg/kg body weight, based on the amount of the siRNA.

In some embodiments, the present disclosure provides a method of inhibiting the expression of PCSK9 gene in hepatocytes, comprising contacting an effective amount of the siRNA and/or the pharmaceutical composition and/or the siRNA conjugate of the present disclosure with the hepatocytes, and introducing the siRNA and/or the pharmaceutical composition and/or the siRNA conjugate of the present disclosure into the hepatocytes, so as to realize the purpose of inhibiting the expression of PCSK9 gene in hepatocytes through the mechanism of RNA interference. The hepatocytes may be selected from hepatoma cell lines (such as SMMC-7721, HepG2 and Huh7), and isolated primary hepatocytes. In some embodiments, the hepatocytes are HepG2 hepatoma cells.

In the case where the expression of PCSK9 gene in a cell is inhibited by the method of the present disclosure, the amount of the siRNA in the modified siRNA and/or the pharmaceutical composition and/or the siRNA conjugate of the present disclosure is generally such an amount that is sufficient to reduce the expression of the target gene and results in an extracellular concentration of 1 pM to 1  $\mu$ M, or 0.01 nM to 100 nM, or 0.05 nM to 50 nM, or 0.05 nM to about 5 nM on the surface of the target cells. The amount required to achieve this topical concentration will vary with various factors, including the delivery method, the delivery site, the number of cell layers between the delivery site and the target cells or tissues, the delivery route (topical or systemic), etc. The concentration at the delivery site may be significantly higher than that on the surface of the target cells or tissues.

### **Kit**

The present disclosure provides a kit comprising an effective amount of at least one of the modified siRNA, the pharmaceutical composition, and the siRNA conjugate of the present disclosure.

In some embodiments, the kit of the present disclosure may provide the modified siRNA in a container. In some embodiments, the kit of the present disclosure may comprise a container containing a pharmaceutically acceptable excipient. In some embodiments, the kit may further comprise other ingredients, such as stabilizers or preservatives. In some embodiments, the kit of the present disclosure may comprise at least one additional therapeutic agent in other container different from the container for providing the modified siRNA of the

present disclosure. In some embodiments, the kit may comprise an instruction for mixing the modified siRNA with pharmaceutically acceptable carriers and/or excipients or other ingredients (if present).

5 In the kit of the present disclosure, the modified siRNA and the pharmaceutically acceptable carrier and/or excipient, as well as the modified siRNA, the pharmaceutical composition, and/or the siRNA conjugate and/or the conjugate, and/or the pharmaceutically acceptable excipient may be provided in any form, such as in a liquid form, a dry form or a lyophilized form. In some embodiments, the modified siRNA and the pharmaceutically acceptable carrier and/or excipient, and the pharmaceutical composition and/or conjugate and optional pharmaceutically acceptable excipient(s) are substantially pure and/or sterilized. In some 10 embodiments, the kit of the present disclosure may provide sterilized water.

Hereinafter, the present disclosure will be further illustrated by way of examples, but will not be limited thereto in any respect.

### 15 **Examples**

Unless otherwise specified, the reagents and culture media used in following examples are all commercially available, and the procedures used such as nucleic acid electrophoresis and real-time PCR are all performed according to the methods described in Molecular Cloning (Cold Spring Harbor Laboratory Press (1989)).

20 When the siRNA or the siRNA conjugate against PCSK9 gene synthesized in the present disclosure or the siRNA or the siRNA conjugate as negative control was used to transfect cells, Lipofectamine™ 2000 (Invitrogen) was used as a transfection reagent. The specific procedures could refer to the instruction provided by the manufacturer.

25 Unless otherwise specified, ratios of reagents provided below are all calculated by volume ratio (v/v).

All the experimental data are expressed as  $\bar{X} \pm \text{SEM}$  (mean  $\pm$  standard error), and the data are analyzed using Graphpad prism 6.0 statistical analysis software. One-way ANOVA is used for analysis and Tukey is used as the analytical method to compare groups to determine whether the difference between the 30 groups is significant. If no significant difference is found using the one-way ANOVA method, where the overall  $P < 0.05$ , a T-test is used for a two-tailed test; where  $p < 0.05$ , there is a significant difference between the two groups.

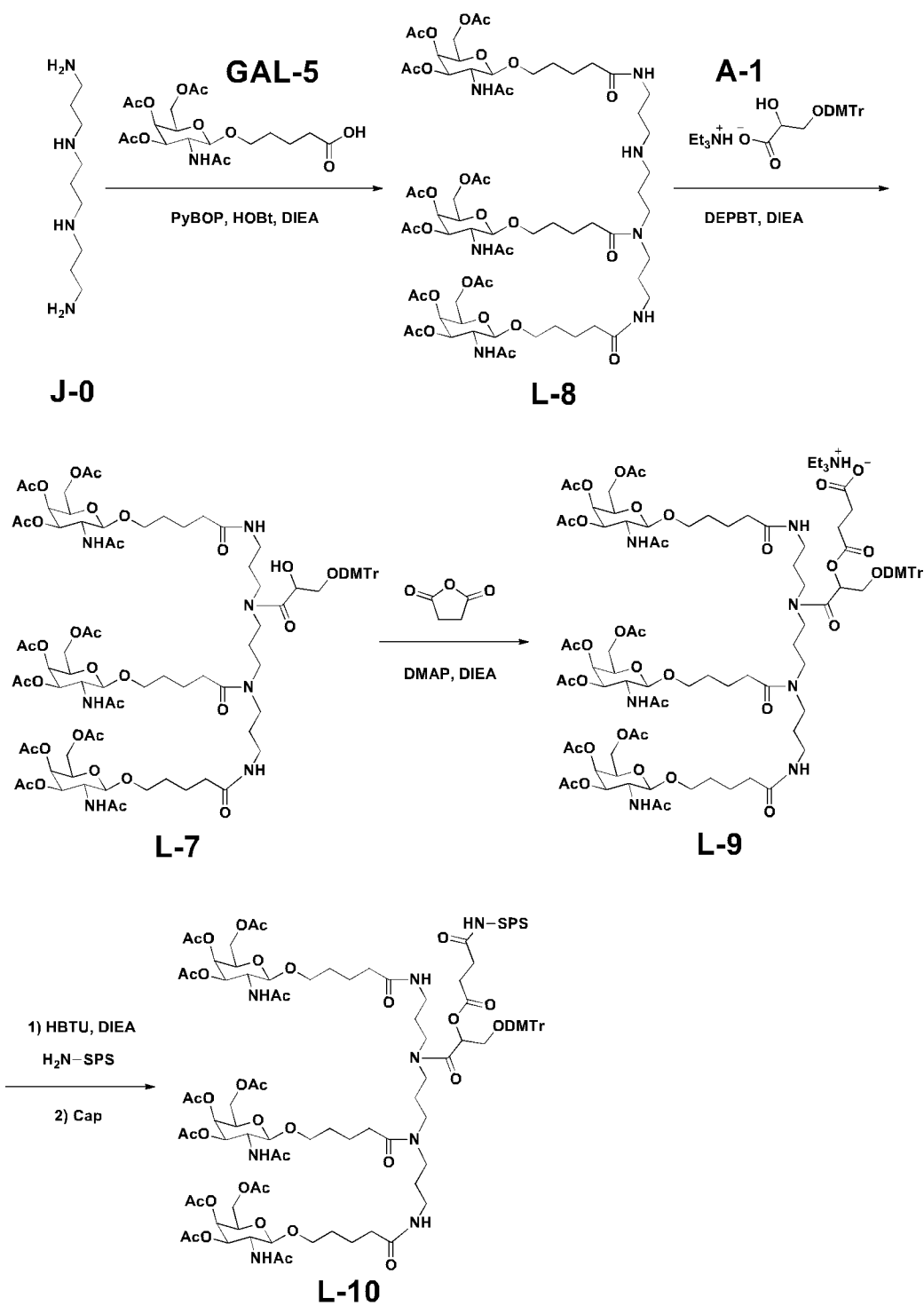
### **Preparation Example 1: The preparation of Conjugate 1**

35 In this preparation example, Conjugate 1 shown in Table 3 was synthesized and its number was L10-siPCSKA1M1S. The conjugated siRNA in this conjugate has the sense strand and antisense strand sequences of Conjugate 1 in Table 3.

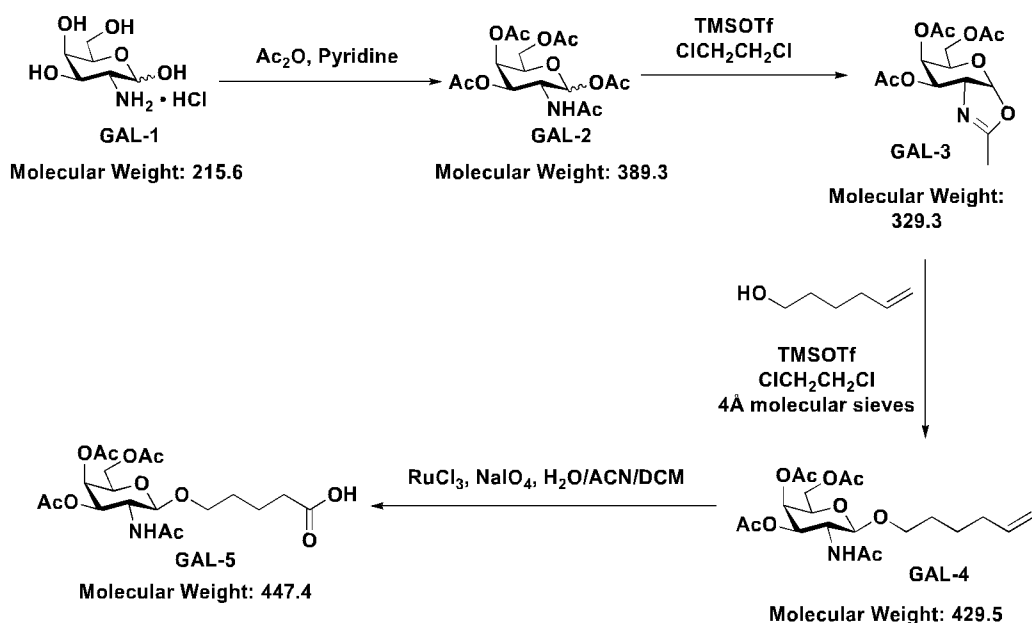
(1-1) Synthesis of Compound L-10:

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Compound L-10 was synthesized according to the following method:



(1-1-1) Synthesis of GAL-5 (a terminal segment of the conjugation molecule)



## (1-1-1a) Synthesis of GAL-2

100.0 g of GAL-1 (N-acetyl-D-galactosamine hydrochloride, CAS No.: 1772-03-8, purchased from NingBo hongxiang bio-chem Co., Ltd., 463.8 mmol) was dissolved in 1000 ml of anhydrous pyridine, to which 540 ml of acetic anhydride (purchased from Enox Inc., 5565.6 mmol) was added in an ice water bath to react under stirring at room temperature for 1.5 hours. The resultant reaction solution was poured into 10 L of ice water and subjected to suction filtration under reduced pressure. The residue was washed with 2 L of ice water, and then added with a mixed solvent of acetonitrile/toluene (v/v ratio of acetonitrile: toluene = 1:1) until completely dissolved. The solvent was removed by evaporation to give 130.0 g of product GAL-2 as a white solid.

## (1-1-1b) Synthesis of GAL-3

GAL-2 (35.1 g, 90.0 mmol) obtained in step (1-1-1a) was dissolved in 213 ml of anhydrous 1,2-dichloroethane, to which 24.0 g of trimethylsilyl trifluoromethanesulfonate (TMSOTf, CAS No.: 27607-77-8, purchased from Macklin Inc., 108.0 mmol) was added in an ice water bath under nitrogen atmosphere to react at room temperature overnight.

The reaction solution was added with 400 ml dichloromethane for dilution, filtered with diatomite, and then added with 1L saturated aqueous sodium bicarbonate solution and stirred evenly. An organic phase was isolated. The aqueous phase remained was extracted twice, each with 300 ml of dichloroethane. The organic phases were combined and washed with 300 ml of saturated aqueous sodium bicarbonate solution and 300 ml of saturated brine, respectively. The organic phase was isolated and dried with anhydrous sodium sulfate. The solvent was removed by evaporation to dryness under reduced pressure to give 26.9 g of product GAL-3 as a light yellow viscous syrup.

## (1-1-1c) Synthesis of GAL-4

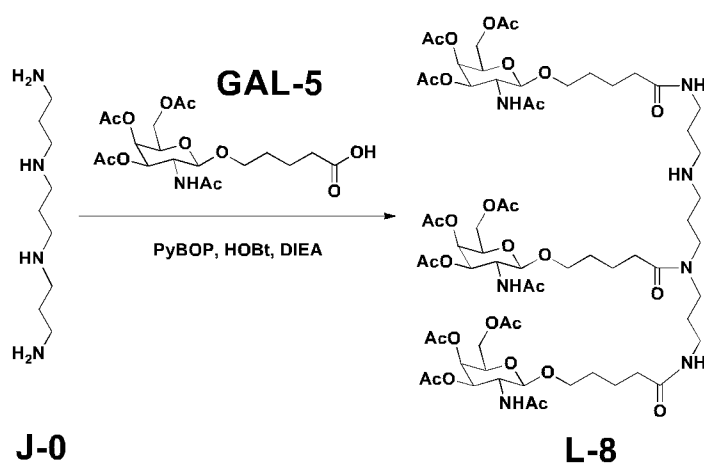
GAL-3 (26.9 g, 81.7 mmol) obtained in step (1-1-1b) was dissolved in 136 ml of anhydrous 1,2-dichloroethane, added with 30 g of dry 4Å molecular sieve powder followed by 9.0 g of 5-hexen-1-ol (CAS No.: 821-41-0, purchased from Adamas-beta Inc., 89.9 mmol), and stirred at room temperature for 30 minutes. 9.08 g of

TMSOTf (40.9 mmol) was added in an ice bath under nitrogen atmosphere to react under stirring at room temperature overnight. The 4Å molecular sieve powder was removed by filtration. The filtrate was added with 300 ml dichloroethane for dilution, filtered with diatomite, and then added with 500 ml of saturated aqueous sodium bicarbonate solution and stirred for 10 minutes for washing. An organic phase was isolated. The aqueous phase was extracted once with 300 ml of dichloroethane. The organic phases were combined and washed with 300 ml of saturated aqueous sodium bicarbonate solution and 300 ml of saturated brine, respectively. The organic phase was isolated and dried with anhydrous sodium sulfate. The solvent was removed by evaporation to dryness under reduced pressure to give 41.3g of product GAL-4 as a yellow syrup, which was directly used in the next oxidation reaction without purification.

#### (1-1-d) Synthesis of GAL-5

GAL-4 (14.9 g, 34.7 mmol) obtained according to the method described in step (1-1-1c) was dissolved in a mixed solvent of 77 ml of dichloromethane and 77 ml of acetonitrile, added with 103 ml of deionized water and 29.7 g of sodium periodate (CAS No.: 7790-28-5, purchased from Aladdin Inc., 138.8 mmol) respectively, and stirred in an ice bath for 10 minutes. Ruthenium trichloride (CAS No.: 14898-67-0, available from Energy Chemical, 238 mg, 1.145 mmol) was added to react at room temperature overnight. The resultant reaction solution was diluted by adding 300 ml of water under stirring, and adjusted to a pH of about 7.5 by adding saturated sodium bicarbonate. The organic phase was isolated and discarded. The aqueous phase was extracted three times, each with 200 ml of dichloromethane, and the organic phase was discarded. The aqueous phase was adjusted to a pH of about 3 with citric acid solids and extracted three times, each with 200 ml of dichloromethane, and the resultant organic phases were combined and dried with anhydrous sodium sulfate. The solvent was removed by evaporation to dryness under reduced pressure to give 6.85 g of product GAL-5 as a white foamy solid. <sup>1</sup>H NMR (400 MHz, DMSO) δ 12.01 (br, 1H), 7.83 (d, J = 9.2 Hz, 1H), 5.21 (d, J = 3.2 Hz, 1H), 4.96 (dd, J = 11.2, 3.2 Hz, 1H), 4.49 (d, J = 8.4 Hz, 1H), 4.07 – 3.95 (m, 3H), 3.92 – 3.85 (m, 1H), 3.74 – 3.67 (m, 1H), 3.48 – 3.39 (m, 1H), 2.20 (t, J = 6.8 Hz, 2H), 2.11 (s, 3H), 2.00 (s, 3H), 1.90 (s, 3H), 1.77 (s, 3H), 1.55 – 1.45 (m, 4H).

#### (1-1-2) Synthesis of L-8

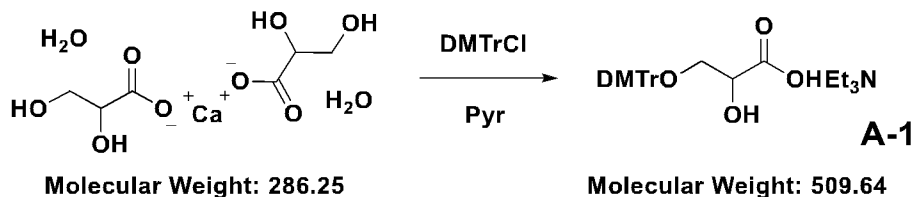


J-0 (9.886 g, 52.5 mmol, purchased from Alfa Aesar Inc.) and GAL-5 (72.819 g, 162.75 mmol, obtained by combining several batches of products) obtained in step (1-1-1) were dissolved in 525 ml of dichloromethane, and added with diisopropylethylamine (DIEA, 44.782 g, 346.50 mmol), benzotriazol-1-yl-oxytripyrrolidino

phosphonium hexafluorophosphate (PyBOP, 90.158 g, 173.25 mmol) and hydroxybenzotriazole (HOBt, 23.410 g, 173.25 mmol) to react at room temperature for 4 hours. The resultant reaction solution was washed by adding 20 ml of saturated sodium bicarbonate solution and 200 ml of saturated brine. The aqueous phase was extracted twice, each with 100 ml of dichloromethane. The organic phases were combined, dried with anhydrous sodium sulfate, and filtered. Then the solvent was removed by evaporation to dryness under reduced pressure to give a crude product. The crude product was purified by using a normal phase silica gel column (200-300 mesh). The column was added with 10 wt% triethylamine for neutralizing the acidity of silica gel, equilibrated with 1 wt% triethylamine, and eluted with a gradient elution of dichloromethane: methanol = 100:25-100:40. The eluate was collected, and the solvent was removed by evaporation to dryness under reduced pressure to give 38.8 g of pure product L-8. <sup>1</sup>H NMR (400 MHz, DMSO) δ 7.84 (d, J = 9.0 Hz, 3H), 7.27 - 7.23 (m, 1H), 7.13 - 7.18 (m, 1H), 5.22 (d, J = 3.1 Hz, 3H), 4.97 (dd, J = 11.3, 3.1 Hz, 3H), 4.48 (d, J = 8.4 Hz, 3H), 4.09 - 3.98 (m, 9H), 3.88 (dd, J = 19.3, 9.3 Hz, 3H), 3.75 - 3.66 (m, 3H), 3.44 - 3.38 (m, 3H), 3.17 - 3.30 (m, 4H), 3.10 - 2.97 (m, 4H), 2.35 - 2.20 (m, 6H), 2.15 - 2.08 (m, 9H), 2.07 - 1.98 (m, 13H), 1.94 - 1.87 (m, 9H), 1.81 - 1.74 (m, 9H), 1.65 - 1.42 (m, 18H). MS m/z: C<sub>85</sub>H<sub>119</sub>N<sub>7</sub>O<sub>30</sub>, [M+H]<sup>+</sup>, calculated: 1477.59, measured: 1477.23.

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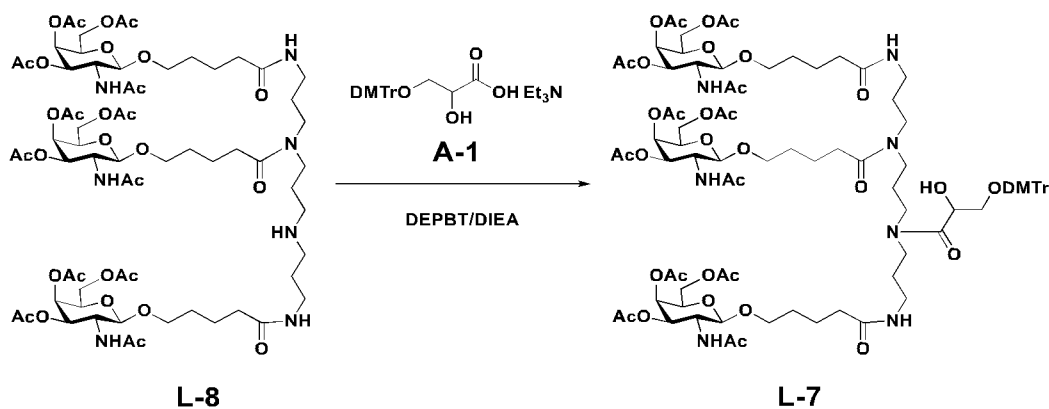
(1-1-3a) Synthesis of A-1



DMTrCl (4,4'-dimethoxytrityl chloride, 101.65 g, 300 mmol) was dissolved in 1000 ml of anhydrous pyridine, and added with calcium DL-glycerate hydrate (28.63 g, 100 mmol) to react at 45°C for 20 hours. The resultant reaction solution was filtered. The residue was rinsed with 200 ml of DCM, and the filtrate was concentrated to dryness under reduced pressure. The residue was redissolved in 500 ml of dichloromethane and washed twice, each with 200 ml of 0.5 M triethylamine phosphate (pH = 7-8). The aqueous phase was extracted twice, each with 200 ml of dichloromethane. The organic phases were combined, dried with anhydrous sodium sulfate, and filtered. The solvent was removed by evaporation to dryness under reduced pressure, and the residue was purified by using a normal phase silica gel column (200-300 mesh). The column was eluted with a gradient elution of petroleum ether: ethyl acetate: dichloromethane: methanol = 1:1:1:0.35 - 1:1:1:0.55. The eluate was collected, and the solvent was removed by evaporation to dryness under reduced pressure. The residue was redissolved in 600 ml of dichloromethane, and washed once with 200 ml of 0.5 M triethylamine phosphate. The aqueous phase was extracted once with 200 ml of dichloromethane. The organic phases were combined, dried with anhydrous sodium sulfate, and filtered. The solvent was removed by evaporation to dryness under reduced pressure, and the residue was dried under reduced pressure in a vacuum oil pump overnight to give 50.7 g of product A-1 as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 7.46 (ddd, J = 6.5, 2.3, 1.1 Hz, 1H), 7.40 - 7.28 (m, 7H), 6.89 - 6.81 (m, 4H), 4.84 (d, J = 5.0 Hz, 1H), 4.36 - 4.24 (m, 1H), 4.29 (s, 6H), 3.92 (dd, J = 12.4, 7.0 Hz, 1H), 3.67 (dd, J = 12.3, 7.0 Hz, 1H), 2.52 (q, J = 6.3 Hz, 6H), 1.03 (t, J = 6.3 Hz, 9H). MS m/z: C<sub>24</sub>H<sub>23</sub>O<sub>6</sub>, [M-H]<sup>-</sup>, calculated: 407.15, measured: 406.92.

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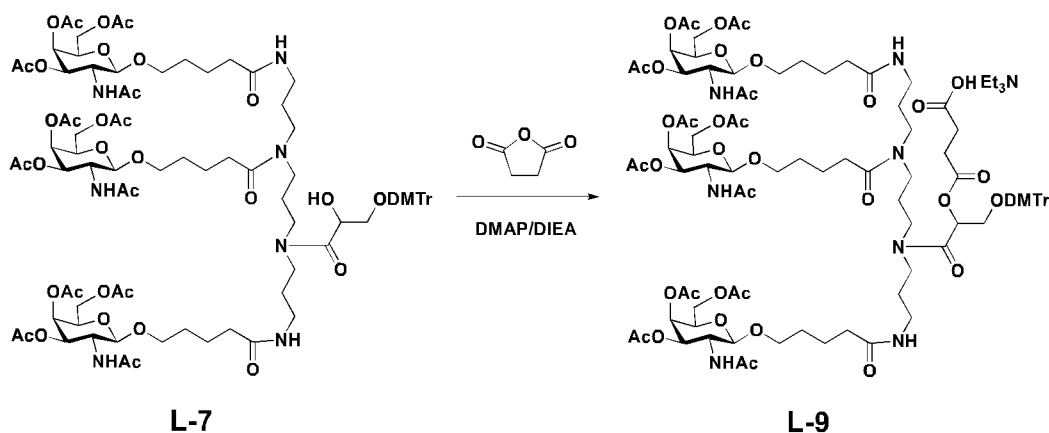
(1-1-3b) Synthesis of L-7



L-8 (40 g, 27.09 mmol, obtained by combining several batches of products) obtained in step (1-1-2) and A-1 (41.418 g, 81.27 mmol) obtained in step (1-1-3a) were mixed and dissolved in 271 ml of dichloromethane, added with 3-diethoxyphosphoryl-1,2,3-benzotrizin-4(3H)-one (DEPBT) (24.318 g, 81.37 mmol), and further  
 5 added with diisopropylethylamine (21.007 g, 162.54 mmol) to react under stirring at 25°C for 1.5 hours. The organic phase was washed with 800 ml of saturated sodium bicarbonate. The aqueous phase was extracted three times, each with 50 ml of dichloromethane. The organic phase was washed with 150 ml of saturated brine, and the aqueous phase was extracted once with 50 ml of dichloromethane, and the organic phases were combined,  
 10 dried with anhydrous sodium sulfate and filtered. The solvent was removed by evaporation to dryness under reduced pressure, and the residue was foam-dried in a vacuum oil pump overnight to give a crude product. The crude product was subjected to a column purification. The column was filled with 2 kg normal phase silica gel (200-300 mesh), added with 200 ml triethylamine for neutralizing the acidity of silica gel, equilibrated with petroleum ether containing 1 wt% triethylamine, and eluted with a gradient elution of petroleum ether: ethyl acetate: dichloromethane: N,N-dimethylformamide = 1:1:1:0.5 - 1:1:1:0.6. The eluate was collected, and the  
 15 solvent was removed by evaporation to dryness under reduced pressure to give 40.4 g of pure product L-7. <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  7.90 – 7.78 (m, 4H), 7.75 – 7.64 (m, 1H), 7.38 – 7.18 (m, 9H), 6.91 – 6.83 (m, 4H), 5.25 – 5.10 (m, 4H), 4.97 (dd,  $J$  = 11.2, 3.2 Hz, 3H), 4.48 – 4.30 (m, 4H), 4.02 (s, 9H), 3.93 – 3.84 (m, 3H), 3.76 – 3.66 (m, 9H), 3.45 – 3.35 (m, 3H), 3.24 – 2.98 (m, 10H), 2.30 – 2.20 (m, 2H), 2.11 – 1.88 (m, 31H), 1.80 – 1.40 (m, 28H). MS  $m/z$ : C<sub>90</sub>H<sub>128</sub>N<sub>7</sub>O<sub>35</sub>, [M-DMTr]<sup>+</sup>, calculated: 1564.65, measured: 1564.88.

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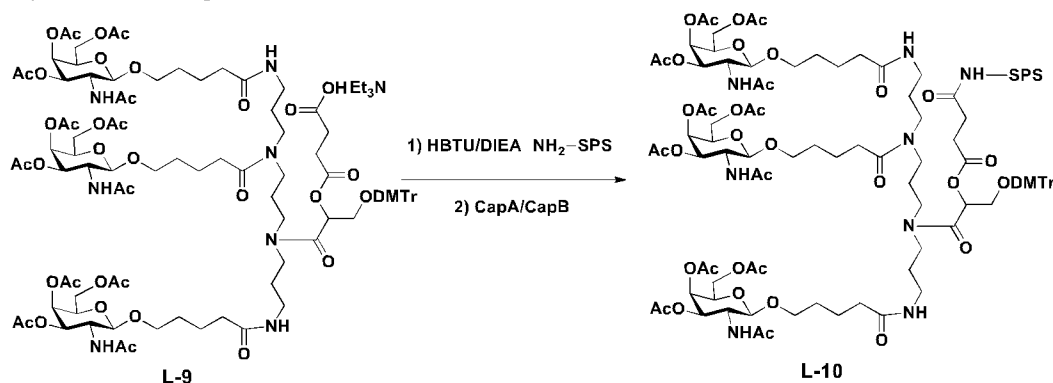
(1-1-4) Synthesis of L-9



L-7 (40g, 21.4247mmol) obtained in step (1-1-3b), succinic anhydride (4.288g, 42.8494mmol) and 4-dimethylaminopyridine (DMAP, 5.235g, 42.8494mmol) were mixed and dissolved in 215 ml of

dichloromethane, further added with diisopropylethylamine (DIEA, 13.845g, 107.1235mmol), and stirred at 25°C for 24 hours. The resultant reaction solution was washed with 800 ml of 0.5 M triethylamine phosphate. The aqueous phase was extracted three times, each with 5 ml of dichloromethane. The organic phases were combined and evaporated to dryness under reduced pressure to give a crude product. The crude product was subjected to a column purification. The column was filled with 1 kg normal phase silica gel (200-300 mesh), added with 1 wt% triethylamine for neutralizing the acidity of silica gel, equilibrated with dichloromethane, and eluted with a gradient elution of 1wt% triethylamine in dichloromethane: methanol = 100:18-100:20. The eluate was collected, and the solvent was removed by evaporation to dryness under reduced pressure to give 31.0 g of pure product L-9 conjugation molecule. <sup>1</sup>H NMR (400 MHz, DMSO) δ 8.58 (d, *J* = 4.2 Hz, 1H), 7.94 – 7.82 (m, 3H), 7.41 – 7.29 (m, 5H), 7.22 (d, *J* = 8.1 Hz, 5H), 6.89 (d, *J* = 8.3 Hz, 4H), 5.49 – 5.37 (m, 1H), 5.21 (d, *J* = 3.0 Hz, 3H), 4.97 (d, *J* = 11.1 Hz, 3H), 4.49 (d, *J* = 8.2 Hz, 3H), 4.02 (s, 9H), 3.88 (dd, *J* = 19.4, 9.4 Hz, 3H), 3.77 – 3.65 (m, 9H), 3.50 – 3.39 (m, 6H), 3.11 – 2.90 (m, 5H), 2.61 – 2.54 (m, 4H), 2.47 – 2.41 (m, 2H), 2.26 – 2.17 (m, 2H), 2.15 – 1.95 (m, 22H), 1.92 – 1.84 (m, 9H), 1.80 – 1.70 (m, 10H), 1.65 – 1.35 (m, 17H), 1.31 – 1.19 (m, 4H), 0.96 (t, *J* = 7.1 Hz, 9H). MS *m/z*: C<sub>94</sub>H<sub>132</sub>N<sub>7</sub>O<sub>38</sub>, [M-DMTr]<sup>+</sup>, calculated: 1664.72, measured: 1665.03.

#### (1-1-5) Synthesis of Compound L-10



In this step, Compound L-10 was prepared by linking the L-9 conjugation molecule to a solid phase support.

The L-9 conjugation molecule (22.751 g, 11 mmol) obtained in step (1-1-4), O-benzotriazol-tetramethyluronium hexafluorophosphate (HBTU, 6.257 g, 16.5 mmol) and diisopropylethylamine (DIEA, 2.843 g, 22 mmol) were mixed and dissolved in 900 ml of acetonitrile, and stirred at room temperature for 5 minutes. The resultant reaction solution was added with Aminomethyl resin (H<sub>2</sub>NResin, 88 g, 100-200 mesh, amino loading: 400 μmol/g, purchased from Tianjin Nankai HECHENG S&T Co., Ltd.). A reaction was performed on a shaker at 25°C and at a rotation speed of 150 rpm/min for 18 hours, followed by filtration. The residue was rinsed twice (each with 300 ml of DCM) and three times (each with 300 ml of acetonitrile), and dried in a vacuum oil pump for 18 hours. Then starting materials (CapA, CapB, 4-dimethylaminopyridine (DMAP) and acetonitrile) were added according to the charge ratio as shown in Table 2 for a capping reaction. The reaction was performed on a shaker at 25°C and at a rotation speed of 150 rpm/min for 5 hours. The reaction liquid was filtered. The residue was rinsed three times, each with 300 ml of acetonitrile. The solvent was removed by evaporation to dryness under reduced pressure, and the residue was dried under reduced pressure in a vacuum oil pump overnight to give 102 g of Compound L-10 (i.e., the L-9 conjugation molecule linked to a solid phase support), with a loading of 90.8 μmol/g.

Table 2 The charge ratio of capping reaction

| Starting Materials | Amount  | Specs              | Lot No.   | Manufacturer             |
|--------------------|---------|--------------------|-----------|--------------------------|
| CapA               | 1980 ml | ——                 | ——        | ——                       |
| CapB               | 220 ml  | ——                 | ——        | ——                       |
| DMAP               | 1.100 g | analytical pure    | I1422139  | Aladdin                  |
| Acetonitrile       | 220 ml  | spectroscopic pure | O15161001 | CINC (Shanghai) Co., Ltd |

In the above table, Cap A and Cap B are solutions of capping agents. Cap A is a mixed solution of 20% by volume of N-methylimidazole in pyridine/acetonitrile, wherein the volume ratio of pyridine to acetonitrile is 3:5. Cap B is a solution of 20% by volume of acetic anhydride in acetonitrile.

#### (1-2) Synthesis of a sense strand of Conjugate 1

A sense strand of siRNA Conjugate 1 in Table 3 was synthesized by linking nucleoside monomers one by one in 3' to 5' direction according to the arrangement sequence of nucleotides in the sense strand by the phosphoramidite solid phase synthesis method, starting the cycles from the Compound L-10 prepared in the above step. The linking of each nucleoside monomer included a four-step reaction of deprotection, coupling, capping, and oxidation or sulfurization. Therein, when two nucleotides are linked via a phosphoester bond, a four-step reaction of deprotection, coupling, capping, and oxidation was included during linking of the later nucleoside monomer; and when two nucleotides is linked via a phosphorothioate linkage, a four-step reaction of deprotection, coupling, capping, and sulfurization was included during linking of the later nucleoside monomer. The synthesis conditions are as follows.

The nucleoside monomers are provided in a 0.1 M acetonitrile solution. The condition for deprotection reaction in each step is identical, i.e., a temperature of 25°C, a reaction time of 70 seconds, a solution of dichloroacetic acid in dichloromethane (3% v/v) as a deprotection reagent, and a molar ratio of dichloroacetic acid to the protecting group 4,4'-dimethoxytrityl on the solid phase support of 5:1.

The condition for coupling reaction in each step is identical, including a temperature of 25°C, a molar ratio of the nucleic acid sequence linked to the solid phase support to nucleoside monomers of 1:10, a molar ratio of the nucleic acid sequence linked to the solid phase support to a coupling reagent of 1:65, a reaction time of 600 seconds, and 0.5 M acetonitrile solution of 5-ethylthio-1H-tetrazole (ETT) as a coupling reagent.

The condition for capping reaction in each step is identical, including a temperature of 25°C, a reaction time of 15 seconds, a mixed solution of Cap A and Cap B in a molar ratio of 1:1 as a solution of capping agent, and a molar ratio of the capping agent to the nucleic acid sequence linked to the solid phase support of 1:1:1 (acetic anhydride: N-methylimidazole: the nucleic acid sequence linked to the solid phase support).

The condition for oxidation reaction in each step is identical, including a temperature of 25°C, a reaction time of 15 seconds, and 0.05 M iodine water as an oxidation reagent; and a molar ratio of iodine to the nucleic acid sequence linked to the solid phase support in the coupling step of 30:1. The reaction is carried out in a mixed solvent of tetrahydrofuran: water: pyridine (3:1:1).

The condition for sulfurization reaction in each step is identical, including a temperature of 25°C, a reaction time of 300 seconds, and xanthane hydride as a sulfurization reagent; and a molar ratio of the sulfurization reagent to the nucleic acid sequence linked to the solid phase support in the coupling step of 120:1. The reaction is carried out in a mixed solvent of acetonitrile: pyridine (1:1).

After the linking of the last nucleoside monomer was completed, the nucleic acid sequence linked to the solid phase support was cleaved, deprotected, purified, desalted, and then lyophilized to obtain the sense strand, wherein,

5

The conditions for cleavage and deprotection are as follows: adding the synthesized nucleotide sequence linked to the support into 25 wt% aqueous ammonia to react at 55°C for 16 hours, wherein the amount of the aqueous ammonia is 0.5 ml/μmol. The remaining support was removed by filtration, and the supernatant was concentrated to dryness in vacuum.

10

The conditions for purification and desalination are as follows: purification of the nucleic acid is achieved by using a preparative ion chromatography purification column (Source 15Q) with a gradient elution of NaCl. Specifically, eluent A is 20 mM sodium phosphate (pH 8.1), solvent is water/acetonitrile in 9:1 (v/v); eluent B is 1.5 M sodium chloride, 20 mM sodium phosphate (pH 8.1), solvent is water/acetonitrile in 9:1 (v/v); elution gradient: the ratio of eluent A: eluent B = 100:0-50:50. The eluate is collected, combined and desalted by using a reverse phase chromatography purification column. The specific condition includes: using a Sephadex column for desalination (filler: Sephadex G25) and eluting with deionized water.

15

The detection method is as follows: the purity of the aforementioned sense strand was determined by ion exchange chromatography (IEX-HPLC); and the molecular weight was analyzed by Liquid Chromatography-Mass Spectrometry (LC-MS). The fact that the measured values were in conformity with the calculated values indicates that the sense strand SS of which 3' terminal was conjugated to the L-9 conjugation molecule was synthesized.

20

#### 25 (1-3) Synthesis of an antisense strand of Conjugate 1

An antisense strand of Conjugate 1 in Table 3 was synthesized by the phosphoramidite solid phase synthesis method, starting the cycles from a universal solid phase support (UnyLinker™ loaded NittoPhase®HL Solid Supports, Kinovate Life Sciences Inc.). The reaction conditions of deprotection, coupling, capping, oxidation or sulfurization, cleavage and deprotection, and purification and desalting in the solid phase synthesis method were the same as those used for the synthesis of the sense strand. Then, lyophilization was performed to obtain an antisense strand. The purity of the antisense strand was detected by ion exchange chromatography (IEX-HPLC); and the molecular weight was analyzed by liquid chromatography-mass spectrometry (LC-MS). The result that the measured values were in conformity with the calculated values indicates that the antisense strands AS having the target sequences were synthesized.

30

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#### (1-4) Synthesis of Conjugate 1

For Conjugate 1, the sense strand and antisense strand were respectively dissolved in water for injection to give a solution of 40 mg/mL. They were mixed in an equimolar ratio, heated at 50°C for 15 min, cooled at room temperature to produce an annealed product, and then lyophilized to give a lyophilized powder. After the conjugate was diluted to a concentration of 0.2 mg/mL with ultra-pure water (Milli-Q ultra-pure water instrument, with resistivity of 18.2MΩ\*cm (25°C)), the molecular weight was determined by a liquid chromatography-mass spectrometry (LC-MS) instrument (purchased from Waters Corp., model: LCT Premier). The fact that the measured values were in conformity with the calculated values indicates that the synthesized Conjugate 1 was the designed target double-stranded nucleic acid sequence with the L-9 conjugation molecule. Conjugate 1 has the structure as shown by Formula (403). The conjugated siRNA in this conjugate has the sequences of Conjugate 1 (also referred to as L10-siPCSKa1M1S) as shown in Table 3.

40

45

**Preparation Example 2: The preparations of Conjugates 2-7**

Conjugates 2-7 as shown in Table 3 were prepared by the same method as in Preparation Example 1 except that the sense strand and antisense strand sequences involved in the synthesis were the sequences of the sense strand and antisense strand of the conjugated siRNA in Conjugate 2, Conjugate 3, Conjugate 4, Conjugate 5, Conjugate 6, or Conjugate 7 as shown in Table 3. Thus, Conjugates 2-7 were obtained respectively. The molecular weights of conjugates 2-7 were determined.

Table 3 lists the numbers and the siRNA sequences of the conjugates.

Table 3: siRNA conjugates

| Conjugates  | No.             | Sequence direction 5'-3' |                                                | SEQ ID NO |
|-------------|-----------------|--------------------------|------------------------------------------------|-----------|
| Conjugate 1 | L10-siPCSKa1M1S | Sense strand             | AmsAmsGmCmAmAmGfCfAfGmAmCmAmUmUmUmAmUmCm       | 25        |
|             |                 | Antisense strand         | GmsAfsUmAmAmAfUmGmUmCmUmGmCmUfUmGfCmUmUmsGmsGm | 26        |
| Conjugate 2 | L10-siPCSKb1M1S | Sense strand             | UmsUmsUmGmUmAmGfCfAfUmUmUmUmUmAmUmAmAm         | 85        |
|             |                 | Antisense strand         | UmsUfsAmAmUmAfAmAmAmAmUmGfCmUfAmCmAmAmAmsAmsCm | 86        |
| Conjugate 3 | L10-siPCSKc1M1S | Sense strand             | GmsCmsCmUmGmGmAfGfUfUmUmAmUmUmCmGmGmAmAm       | 145       |
|             |                 | Antisense strand         | UmsUfsCmCmGmAfAmUmAmAmAmCmUmCfCmAfGmGmCmsCmsUm | 146       |
| Conjugate 4 | L10-siPCSKd1M1S | Sense strand             | CmsUmsGmUmUmUmUfGfCfUmUmUmUmGmUmAmAmCmUm       | 205       |
|             |                 | Antisense strand         | AmsGfsUmUmAmCfAmAmAmAmGmCmAmAfAmAfCmAmGmsGmsUm | 206       |
| Conjugate 5 | L10-siPCSKe1M1S | Sense strand             | GmsGmsUmUmUmUmGfUfAfGmCmAmmUmUmUmUmUmAmUm      | 265       |
|             |                 | Antisense strand         | AmsUfsAmAmAmAfAmUmGmCmUmAmCmAfAmAfAmCmCmsCmsAm | 266       |
| Conjugate 6 | L10-siPCSKf1M1S | Sense strand             | GmsUmsGmAmCmUmUfUfUfUmAmAmAmAmUmAmAmAm         | 325       |
|             |                 | Antisense strand         | UmsUfsUmUmAmUfUmUmUmAmAmAmAmAfGmUfCmAmCmsCmsAm | 326       |
| Conjugate 7 | L10-siPCSKg4M5S | Sense strand             | CmsUmsAmGmAmCmCfUmGfUmdTUmUmGmCmUmUmUmUmGmUm   | 397       |

|  |  |                         |                                                        |     |
|--|--|-------------------------|--------------------------------------------------------|-----|
|  |  | <b>Antisense strand</b> | AmsCfsAmAfAfAfGmCfAmAfAmAmC<br>mAfGmGfUmCfUmAmGmsAmsAm | 398 |
|--|--|-------------------------|--------------------------------------------------------|-----|

### Preparation Example 3: The synthesis of the siRNA sequences

SiRNA 1 as shown in Table 3 was synthesized by the same method as in Preparation Example 1 except that

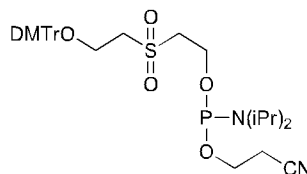
5

1) as to the **sense strand**, the cycles was started from a universal solid phase support (UnyLinker™ loaded NittoPhase®HL Solid Supports, Kinovate Life Sciences Inc.);

10

2) as to the **antisense strand**, the sequence of siRNA 1 differed from that of the antisense strand of the conjugated siRNA in Conjugate 1 in that the antisense strand of siRNA 1 had 5'-phosphate at the first nucleotide at 5' terminal. Therefore, during preparation of the antisense strands according to the phosphoramidite solid phase synthesis method, after the linking of the last nucleoside monomer of the antisense strand, the monomer of Formula (CPR-I) (purchased from Suzhou GenePharma Inc. as Cat#13-2601-XX) was linked to 5' terminal of the antisense strand by a four-step reaction of deprotection, coupling,

15



(CPR-I)

20 During the linking, the conditions of deprotection, coupling, capping, oxidation, cleavage and deprotection, purification and desalting used were the same as those used in the synthesis of the sense strand.

SiRNA 2 was prepared by using the same method as that for preparing siRNA 1 except that the nucleic acid sequences of the sense strand and antisense strand of the siRNA involved in the synthesis of siRNA 2 were the sense strand and antisense strand sequences of siRNA 2 as shown in Table 4.

25

SiRNAs 3-6 were prepared by using the same method as that for preparing siRNA 1 except that the nucleic acid sequences of the sense strand and antisense strand of the siRNA involved in the synthesis were the sense strand and antisense strand sequences of siRNA 3, siRNA 4, siRNA 5 or siRNA 6 as shown in Table 4, wherein the antisense strands of these siRNAs had a 5'-phosphate modification at the first nucleotide at 5' terminal. Therefore, an antisense strand with a 5'-phosphorothioate modification can be prepared by replacing the oxidation reaction condition with a sulfurization reaction condition in the linking of the CPR-I monomer. Thus, siRNA 3, siRNA 4, siRNA 5 or siRNA 6 was obtained respectively.

35 Table 4 lists the numbers and the sequences of the siRNAs.

Table 4: siRNA sequences

| siRNA   | No.           | Sequence direction 5'-3' |                                                      | SEQ ID NO |
|---------|---------------|--------------------------|------------------------------------------------------|-----------|
| siRNA 1 | siPCSKa1M1SP  | Sense strand             | AmsAmsGmCmAmAmGfCfAfGmAmCmA<br>mUmUmUmAmUmCm         | 361       |
|         |               | Antisense strand         | PGmsAfsUmAmAmAfUmGmUmCmUmGm<br>CmUfUmGfCmUmUmsGmsGm  | 362       |
| siRNA 2 | siPCSKb1M1SP  | Sense strand             | UmsUmsUmGmUmAmGfCfAfUmUmUmU<br>mUmAmUmUmAmAm         | 363       |
|         |               | Antisense strand         | PUmsUfsAmAmUmAfAmAmAmAmUmGf<br>CmUfAmCmAmAmAmsAmsCm  | 364       |
| siRNA 3 | siPCSKc1M1SPs | Sense strand             | GmsCmsCmUmGmGmAfGfUfUmUmAmU<br>mUmCmGmGmAmAm         | 365       |
|         |               | Antisense strand         | PsUmsUfsCmCmGmAfAmUmAmAmAmC<br>mUmCfCmAfGmGmCmsCmsUm | 366       |
| siRNA 4 | siPCSKd1M1SPs | Sense strand             | CmsUmsGmUmUmUmUfGfCfUmUmUmU<br>mGmUmAmAmCmUm         | 367       |
|         |               | Antisense strand         | PsAmsGfsUmUmAmCfAmAmAmAmGmC<br>mAmAfAmAfCmAmGmsGmsUm | 368       |
| siRNA 5 | siPCSKe1M1SPs | Sense strand             | GmsGmsUmUmUmUmGfUfAfGmCmAmU<br>mUmUmUmUmAmUm         | 369       |
|         |               | Antisense strand         | PsAmsUfsAmAmAmAfAmUmGmCmUmA<br>mCmAfAmAfAmCmCmsCmsAm | 370       |
| siRNA 6 | siPCSKf1M1SPs | Sense strand             | GmsUmsGmAmCmUmUfUfUfUmAmAmA<br>mAmUmAmAmAmAm         | 371       |
|         |               | Antisense strand         | PsUmsUfsUmUmAmUfUmUmUmAmAmA<br>mAmAfGmUfCmAmCmsCmsAm | 372       |

#### Experimental Example 1 The inhibitory activity and off-target effect of the siRNA in *in vitro* psiCHECK system

- 5 In this experimental example, the on-target activity and off-target effect of the siRNAs of the present disclosure were evaluated by detecting the inhibition of each antisense strand of siRNAs 1-6 against the expression of the on-target plasmid, and the inhibition of each sense strand, seed region of the antisense strand or seed region of the sense strand of the siRNAs against the expression of the off-target plasmid in *in vitro* psiCHECK system.
- 10 According to the method described in Kumico Ui-Tei et. al., Functional dissection of siRNA sequence by systematic DNA substitution: modified siRNA with a DNA seed arm is a powerful tool for mammalian gene silencing with significantly reduced off-target effect. Nucleic Acids Research, 2008.36(7), 2136-2151, plasmids for detection were constructed and co-transfected with the siRNA to be detected into HEK293A cells; and the expression levels of the dual luciferase reporter gene reflect the on-target activity and off-target effect of the
- 15 siRNAs. Specific steps are as follows:

## [1] Construction of plasmids for detection

Four types of plasmids for detection were constructed using psiCHECK™-2 (Promega™) plasmid, in which GSCM represents the on-target plasmid; and PSCM, GSSM and PSSM represent the off-target plasmids:

5

(1) GSCM, used for detecting the on-target activity of the antisense strand of the siRNA, contains a target sequence which contains a region which is completely complementary to the sequence of the antisense strand of the siRNA to be detected, wherein the target sequence in GSCM corresponding to siRNAs 1, 2, 4, 5 and 6 is shown by SEQ ID NO: 373:

10

5'-

GGCGTGCCTGCCAAGCTCACACAGCAGGAAGCTGAGCCAGAAACGCAGATTGGGCTGGCTCT  
GAAGCCAAGCCTCTTCTTACTTCACCCGGCTGGGCTCCTCATTTTTACGGGTAACAGTGAGG  
CTGGGAAGGGGAACACAGACCAGGAAGCTCGGTGAGTGATGGCAGAACGATGCCTGCAGG  
15 CATGGAAGCTTTTTCCGTTATCACCCAGGCCTGATTCACTGGCCTGGCGGAGATGCTTCTAAGG  
CATGGTCGGGGGAGAGGGCCAACAAGTGTCCCTCCTTGAGCACCAGCCCCACCAAGCAAG  
CAGACATTTATCTTTTGGGTCTGTCCTCTCTGTTGCCTTTTTACAGCCAAGCTTTCTAGACCTG  
TTTTGCTTTTGTAAGTGAAGATATTTATCTGGGTTTTGTAGCATTTTTATTAATATGGTGACT  
TTTTAAATAAAAAACAAACAAACGTTGTCCTAACAAAAAAAAAAAAAAAAAAAAAAAAA-3' (SEQ  
20 ID NO: 373);

and the target sequence in GSCM corresponding to siRNA 3 is shown in SEQ ID NO: 374:

5'-

25 GCGTGGCCAAGGGTGCCAGCATGCGCAGCCTGCGCGTGCTCAACTGCCAAGGGAAGGGCAC  
GGTTAGCGGCACCCTCATAGGCCTGGAGTTTATTCGGAAAAGCCAGCTGGTCCAGCCTGTGG  
GGCCACTGGTGGTGCTGCTGCCCCGCGGGTGGGTACAGCCGCGTCCTCAACGCCGCGCTGC  
CAGCGCCTGGCGAGGGCTGGGGTCGTGCTGGTCACCGCTGCCGGCAACTTCCGGGACGATG  
CCTGCCTCTACTCCCCAGCCTCAGCTCCCGAGGTCATCACAGTTGGGGCCACCAATGCCCAA  
30 GACCAGCCGGTGACCCTGGGGACTTTGGGGACCAACTTTGGCCGCTGTGTGGACCTCTTTGC  
CCCAGGGGAGGACATCATTTGGTGCCTCCAGCGACTGCAGCACCTGCTTTGTGTACAGAGTG  
GGACATCACAGGCTGCTGCCCACGTGGCTGGCATTGCAGCCATGATGCTGTCTGCCGAGCCG  
GAGCTCACCTGGCCGAGTTGAGGCAGAGACTGATCCACTTCTCTGCCAAAGATGTCATCAA  
TGAGGCCTGGTTCCCTGAGGACCAGCGGGTACTG-3' (SEQ ID NO: 374).

35

(2) PSCM, used for detecting the off-target effect of the sense strand, contains a target sequence that is exactly the same as the sequence of the antisense strand of the siRNA to be detected, wherein the target sequence in PSCM corresponding to each siRNA is shown in Table 5a:

40 Table 5a The target sequences in PSCM corresponding to the siRNAs

| siRNA   | Target sequences in PSCM (Sequence direction 5'-3') | SEQ ID NO |
|---------|-----------------------------------------------------|-----------|
| siRNA 1 | GATAAATGTCTGCTTGCTTGG                               | 375       |
| siRNA 2 | TTAATAAAAATGCTACAAAAC                               | 376       |
| siRNA 3 | TTCCGAATAAACTCCAGGCCT                               | 377       |
| siRNA 4 | AGTTACAAAAGCAAAACAGGT                               | 378       |

|         |                       |     |
|---------|-----------------------|-----|
| siRNA 5 | ATAAAAATGCTACAAAACCCA | 379 |
| siRNA 6 | TTTTATTTTAAAAAGTCACCA | 380 |

(3) GSSM is used for detecting the off-target effect of the seed region of the antisense strand, wherein the target sequence in GSSM corresponding to each siRNA is shown in Table 5b:

5 Table 5b The target sequences in PSCM corresponding to the siRNAs

| siRNA   | Target sequences in GSSM (Sequence direction 5'-3') | SEQ ID NO |
|---------|-----------------------------------------------------|-----------|
| siRNA 1 | AACCTACCTACTCCATTTATC                               | 381       |
| siRNA 2 | TGGGGTGCTACGGTTTATTAA                               | 382       |
| siRNA 3 | CTTAAGTTCTGGGATTCGGAA                               | 383       |
| siRNA 4 | CAAGTGGGGTAGGTTGTAAC                                | 384       |
| siRNA 5 | GTTTGGGGTGCTAATTTTAT                                | 385       |
| siRNA 6 | GTTGTCAGGGGGCAAATAAAA                               | 386       |

(4) PSSM is used for detecting the off-target effect of the seed region of the sense strand, wherein the target sequence in PSSM corresponding to each siRNA is shown in Table 5c:

10 Table 5c The target sequences in PSSM corresponding to the siRNAs

| siRNA   | Target sequences in PSSM (Sequence direction 5'-3') | SEQ ID NO |
|---------|-----------------------------------------------------|-----------|
| siRNA 1 | TCGCCCCGTGAGGCTTGCTTTT                              | 387       |
| siRNA 2 | GGCCGCCCCCGGCTACAAACA                               | 388       |
| siRNA 3 | GGAATCCGCCCCCTCCAGGCAG                              | 389       |
| siRNA 4 | CTGGCACCCCTCAAAACAGTG                               | 390       |
| siRNA 5 | CGCCCCCGTAGACAAAACCAC                               | 391       |
| siRNA 6 | GGGGCGGGGGCCAAAGTCACAC                              | 392       |

A single copy of the aforementioned target sequences was cloned into the Xho I/Not I site of the psiCHECK™-2 plasmid respectively.

## 15 [2] Cell culture and transfection

### (1) Co-transfection of GSCM and siRNA 1

HEK293A cells (purchased from Nanjing Cobioer Biosciences Co., LTD) were cultured in H-DMEM complete media (Hyclone company) containing 10% fetal bovine serum (FBS, Hyclone company), and 0.2 vol% Penicillin-Streptomycin (Hyclone company) at 37°C in an incubator containing 5% CO<sub>2</sub>/95% air.

HEK293A cells were inoculated in a 96-well plate at  $8 \times 10^3$  cells/well. After 16 hours, the cell growth density reached 70 to 80%. At that time, the H-DMEM complete media in the culture wells were aspirated. An 80 µl Opti-MEM medium (GIBCO company) was added to each well and the culture was continued for 1.5 h.

GSCM detection plasmids were diluted with DEPC water to give a 200 ng/μl detection plasmid working solution; siRNA 1 was prepared with DEPC water into siRNA working solutions at 11 different concentrations of 1000 nM, 333 nM, 111 nM, 37.0 nM, 12.3 nM, 4.12 nM, 1.37 nM, 0.46 nM, 0.15 nM, 0.05 nM and 0.017 nM, respectively.

5

A1-A11 solutions were prepared respectively. Each portion of the A1-A11 solutions contains, in turn, 1 μl of siRNA working solution at one of the aforementioned 11 concentrations, 0.05 μl of detection plasmid working solution (containing 10 ng of detection plasmid) and 10 μl of Opti-MEM medium.

10 B solutions were prepared. Each portion of B solution contains 0.2 μl of Lipofectamine™ 2000 and 10 μl of Opti-MEM medium.

C solutions were prepared. Each portion of C solution contains 0.05 μl of detection plasmid working solution (containing 10 ng of detection plasmid) and 10 μl of Opti-MEM medium

15

One portion of B solution was sequentially mixed with one portion of each of the A1-A11 solutions and one portion of C solution respectively. The mixtures were incubated for 20 min at room temperature to obtain 12 transfection complexes X1-X12. Each transfection complex was prepared in triplicate.

20 The transfection complexes X1-X11 were added respectively to the culture wells in the amount of 20 μl/well and mixed evenly, and three aliquots of the transfection complex at the same siRNA concentration were added respectively to three different culture wells to give co-transfection mixtures at final siRNA concentrations of 10 nM, 3.33 nM, 1.11 nM, 0.37 nM, 0.123 nM, 0.0412 nM, 0.0137 nM, 0.0046 nM, 0.0015 nM, 0.0005 nM and 0.00017 nM (recorded as test groups 1-11).

25

The transfection complexes X12 were added respectively to three other culture wells in the amount of 20 μl/well to obtain transfection mixtures without siRNA (recorded as the control groups).

30 After the co-transfection mixtures containing siRNA and the transfection mixtures without siRNA were transfected in the culture wells for 4 hours, each well was supplemented with 100 μl of H-DMEM complete medium containing 20% FBS. The 96-well plate was placed in a CO<sub>2</sub> incubator and the incubation was continued for 24 hours.

(2) Co-transfection of other siRNAs and plasmids.

35

GSCM and siRNAs 2-6 were co-transfected in the same way as GSCM and siRNA 1, except that siRNA 1 was replaced successively by siRNAs 2-6.

40 PSCM and siRNAs 1-6 were co-transfected in the same way as GSCM and siRNAs 1-6, except that GSCM was replaced successively by PSCM.

GSSM and siRNAs 1-6 were co-transfected in the same way as GSCM and siRNAs 1-6, except that GSCM was replaced successively by GSSM.

45 PSSM and siRNAs 1-6 were co-transfected in the same way as GSCM and siRNAs 1-6, except that GSCM was replaced successively by PSSM.

## [3] Detection

The media in the culture wells were aspirated. 150 µl of mixed solution of Dual-Glo® Luciferase reagent and H-DMEM (in a volume ratio of 1:1) was added to each well, and thoroughly blended. After incubation was performed for 10 minutes at room temperature, 120 µl of the mixed solution was transferred to a 96-well ELISA plate. The chemiluminescence value of Firefly (Fir) in each well of the ELISA plate was read using a Synergy II multimode microplate reader (BioTek company). Then, 60 µl of Dual-Glo® Stop & Glo® reagent was added to each well of the ELISA plate, and thoroughly blended. After incubation was performed at room temperature for 10 minutes, the chemiluminescence value of *Renilla* (Ren) in each well of the ELISA plate was read using the microplate reader according to the arrangement for reading Fir.

The luminescence ratio (Ratio = Ren/Fir) of each well of the ELISA plate was calculated, and the luminescence ratio (Ratio (test) or Ratio (control)) of each test group or control group was the average of the Ratios of the three culture wells. Using the luminescence ratio of the control group as reference, the luminescence ratio of each test group was normalized to obtain the ratio R of Ratio (test)/Ratio (control), which represents the expression level, i.e., the relative residual activity, of the reporter gene *Renilla*. The inhibition rate of siRNA was  $(1-R) \times 100\%$ .

The log (inhibitor) vs. response—Variable slope (four parameters) dose-effect curves were fitted using the nonlinear regression analysis of Graphpad 5.0 software, according to the relative residual activities of the reporter gene *Renilla* in the psiCHECK system after transfection with different siRNAs (siRNA 1, siRNA 2, siRNA 3, siRNA 4, siRNA 5 or siRNA 6). Figures 1A-1F are the dose-effect curves of siRNAs 1-6 respectively, wherein the abscissa represents the logarithm of the concentration of siRNAs (lg nM), the ordinate represents the relative residual activity (%) of *Renilla*, and each dot represents the average of the relative residual activities of *Renilla* in the 3 culture wells of the test group relative to the control group.

The IC<sub>50</sub> value of the siRNA to be tested targeting GSCM was calculated based on the function corresponding to the fitted dose-effect curve. The function is as follows:

$$Y = \text{Bot} + \frac{\text{Top} - \text{Bot}}{1 + 10^{(X' - X) \times \text{HillSlope}}}$$

wherein:

Y is the ratio R, i.e., the relative residual activity (%) of *Renilla*,

X is the logarithm of the concentration of transfected siRNAs,

Bot is the Y value at the bottom of the steady-state stage,

Top is the Y value at the top of the steady-state stage,

X' is the X value at which Y is the median value between the bottom and the top, and HillSlope is the slope of the curve at X'.

When Y=50% the corresponding X<sub>50</sub> value was determined based on the dose-effect curve and the corresponding function. The IC<sub>50</sub> value of each siRNA was calculated (The IC<sub>50</sub> value=10<sup>X<sub>50</sub></sup> (nM)). The IC<sub>50</sub> values and R<sup>2</sup> values are listed in Table 6.

Table 6 IC<sub>50</sub> and R<sup>2</sup> values of siRNAs against GSCM

| siRNA   | No.           | IC <sub>50</sub> | R <sup>2</sup> |
|---------|---------------|------------------|----------------|
| siRNA 1 | siPCSKa1M1SP  | 0.0544nM         | 0.9993         |
| siRNA 2 | siPCSKb1M1SP  | 0.0291 nM        | 0.9979         |
| siRNA 3 | siPCSKc1M1SPs | 0.0341 nM        | 0.9995         |
| siRNA 4 | siPCSKd1M1SPs | 0.0194 nM        | 0.9974         |
| siRNA 5 | siPCSKe1M1SPs | 0.0561 nM        | 0.9956         |
| siRNA 6 | siPCSKf1M1SPs | 0.0400 nM        | 0.9927         |

Table 6 and Figures 1A-1F show that the siRNAs of the present disclosure exhibited relatively high inhibitory activity in *in vitro* HEK293A cells, with the IC<sub>50</sub> values ranging from 0.0194 nM to 0.0561 nM.

5

The dose-effect curves of siRNAs 1-6 against their corresponding PSCM, GSSM and PSSM were obtained one by one using the same method as above except that GSCM was replaced by PSCM, GSSM and PSSM respectively. The results show that each siRNA at each concentration has no inhibitory effect on its corresponding off-target plasmids PSCM, GSSM and PSSM.

10

The above results indicate that the siRNAs of the present disclosure have good targeting specificity, and none of the sense strand, the seed region of the antisense strand and the seed region of the sense strand has obvious off-target effect.

15 Experimental example 2 Evaluation of the efficacy of siRNA conjugates in non-human primates (NHP)

Common cynomolgus monkeys (weighing 2.4-3.1 kg) aged 3 to 4 years old were divided into 2 groups according to their body weights. Each group of 4 cynomolgus monkeys, half of which were male, was injected with a single subcutaneous injection of Conjugate 4 or 5 respectively.

20

Common cynomolgus monkeys (weighing 4.0-5.5 kg) aged 4 to 5 years old were divided into 3 groups according to their body weights. Each group of 3 cynomolgus monkeys, all of which were male, was injected with a single subcutaneous injection of Conjugate 1, 2 or 7 respectively.

25 Each conjugate was prepared with a sterile sodium chloride injection into a 9 mg/ml (based on siRNA; the same applies hereinafter) solution. The volume administered to each animal was 1ml/kg, and the administration dose was 9 mg/kg. The n days before administration was defined as D-n, the day of administration was defined as D1, and the nth day after administration was defined as Dn.

30 (2-1) Detection of PCSK9 mRNA in liver tissue

On D-7 and D15, liver puncture was performed respectively on the cynomolgus monkeys to which Conjugate 1, Conjugate 2, Conjugate 4, Conjugate 5 or Conjugate 7 was administered in each group. Each time, liver tissue measuring about 2×2×8 mm<sup>3</sup> was collected, and stored in a 1.5mL sterile EP tube

containing 1ml of RNAlater preservation solution (Sigma Aldrich company). After placed in a refrigerator at 4°C for 24 hours, it was transferred to -80°C for storage.

After that, the liver tissue was homogenized with a tissue homogenizer. Then the total RNA of the liver tissue was extracted and obtained by Trizol (Thermo Fisher company) according to the procedures for total RNA extraction in the instruction.

For the total RNA extracted from the liver tissue of each animal, 1 µg of the total RNA was taken as the template for reverse transcription, and the reagent provided in the reverse transcription kit Goldenstar™ RT6 cDNA Synthesis Kit (purchased from Beijing Tsingke Biotechnology Co., Ltd., Cat No. TSK301M), in which Goldenstar™ Oligo (dT)<sub>17</sub> was selected as the primer, was used to prepare 20 µl of a reverse transcription reaction system according to the procedures for reverse transcription in the instruction of the kit. The total RNA of each of the aforementioned liver tissue was reverse transcribed. Conditions for reverse transcription were as follows: each reverse transcription reaction system was placed and incubated at 50°C for 50 minutes, then incubated at 85°C for 5 minutes, and finally incubated at 4°C for 30 seconds; after the reaction was complete, 80 µl of DEPC water was added to each reverse transcription reaction system to obtain a cDNA-containing solution.

For each reverse transcription reaction system, 5 µl of the aforementioned cDNA-containing solution was taken as the template for qPCR, and the reagent provided in the NovoStart® SYBR qPCR SuperMix Plus kit (purchased from Novoprotein Scientific Co., Ltd., Cat No. E096-01B) was used to prepare 20 µl of a qPCR reaction system, wherein the sequences of PCR primers used for amplifying the target gene PCSK9 and the internal control gene GAPDH are shown in Table 7, and the final concentration of each primer is 0.25 µM. Each qPCR reaction system was placed on an ABI StepOnePlus Real-Time PCR instrument, and was amplified using the three-step method. The amplification procedures consisted of pre-denaturation at 95°C for 10 minutes, denaturation at 95°C for 30 s, and annealing at 60°C for 30 s, followed by extension at 72°C for 30 s. After repeating the aforementioned process of denaturation, annealing, and extension 40 times, a product W containing the amplified target gene PCSK9 and the amplified internal control gene GAPDH was obtained. The product W was then incubated at 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s. The melting curves of the target gene PCSK9 and the internal control gene GAPDH in the product W were respectively collected using a real-time fluorescent quantitative PCR instrument, and the Ct values of the target gene PCSK9 and the internal control gene GAPDH were obtained.

Table 7 The sequences of the primers

| Genes        | Upstream Primers (in 5' to 3' direction) | Downstream Primers (in 5' to 3' direction) |
|--------------|------------------------------------------|--------------------------------------------|
| Monkey PCSK9 | GAAGGGGAACACAGACCAGG(SEQ ID NO: 393)     | CTCCATCAGGCCACAGTGAA(SEQ ID NO: 394)       |
| Monkey GAPDH | GGGAGCCAAAAGGGTCATCA(SEQ ID NO: 395)     | CGTGGACTGTGGTCATGAGT(SEQ ID NO: 396)       |

The relative quantitative calculation of the expression level of the D15 and D-7 target gene PCSK9 was carried out using the comparative Ct ( $\Delta\Delta Ct$ ) method. The calculation method is as follows:

$$\Delta Ct (D15) = Ct (D15 \text{ target gene}) - Ct (D15 \text{ internal control gene})$$

$$\Delta Ct (D-7) = Ct (D-7 \text{ target gene}) - Ct (D-7 \text{ internal control gene})$$

$$\Delta\Delta Ct (D15) = \Delta Ct (D15) - \Delta Ct (D-7 \text{ average})$$

$$\Delta\Delta Ct (D-7) = \Delta Ct (D-7) - \Delta Ct (D-7 \text{ average})$$

wherein the  $\Delta Ct (D-7 \text{ average})$  is the arithmetic mean of the  $\Delta Ct (D-7)$  of each animal before  
5 administration. Thus, each animal corresponds to a  $\Delta\Delta Ct (D15)$  and a  $\Delta\Delta Ct (D-7)$ .

The expression level of PCSK9 mRNA of D15 in an animal was normalized based on D-7 of this animal,  
wherein the expression level of PCSK9 mRNA of D-7 was defined as 100%;

10 the relative expression level of PCSK9 mRNA =  $2^{(-\Delta\Delta Ct(D15))} \times 100\%$ ;

the inhibition rate of the conjugate against PCSK9 mRNA =  $(1 - 2^{(-\Delta\Delta Ct(D15))}) \times 100\%$ .

For the group whose animals were given the same conjugate, the inhibition rate of the conjugate against  
15 PCSK9 mRNA was the arithmetic mean of the inhibition rates in all the animals of this group.

Table 8 shows the inhibition rate of each conjugate against liver PCSK9 mRNA.

Table 8 Inhibition rate of conjugates against PCSK9 mRNA

| Conjugates  | No.             | inhibition rate % |
|-------------|-----------------|-------------------|
| Conjugate 1 | L10-siPCSKa1M1S | $79 \pm 4$        |
| Conjugate 2 | L10-siPCSKb1M1S | $68 \pm 11$       |
| Conjugate 4 | L10-siPCSKd1M1S | $81 \pm 6$        |
| Conjugate 5 | L10-siPCSKe1M1S | $65 \pm 18$       |
| Conjugate 7 | L10-siPCSKg4M5S | $56 \pm 22$       |

20 As can be seen from Table 8, after single administration of 9 mg/kg, all the siRNA conjugates  
exhibited an inhibition rate of more than 56% against PCSK9 mRNA on day 15 after the  
administration relative to day 7 before the administration, wherein Conjugate 1 and Conjugate 4  
showed the inhibition rates of up to 79% and 81% against PCSK9 mRNA in NHP liver tissue  
respectively.

25

(2-2) Detection of PCSK9 protein content in serum

For the NHP to which Conjugate 4 was administered, a venous blood collection was performed on  
D-15, and D-9 and D1 respectively before the administration, and a venous blood collection was  
30 performed on D3, D8, D14, D22, and D29 respectively after the administration. A blood collection  
was performed weekly thereafter for 18 weeks until D127.

For the NHP to which Conjugate 1 or 7 was administered, a venous blood collection was performed  
on D-14, and D-7 and D1 respectively before the administration, and a venous blood collection  
35 was performed was collected on D4, D8, D15, D22, and D29 respectively after the administration.  
A blood collection was performed weekly thereafter for 12 weeks until D85.

Serum was separated from each of the collected blood samples at room temperature.

(2-2-1) Detection of PCSK9 protein content in serum of cynomolgus monkeys to which Conjugate 4 was administered

The serum collected on D-9, D8, D14, D85, D92, D99, D106 and D120 after administration of Conjugate 4 was selected, the aforementioned serum obtained at each time point was diluted by 25 times with the Calibrator Diluent RD5P (1:5 dilution) reagent in the Human Proprotein Convertase 9/PCSK9 Quantikine ELISA Kit (R&D Systems company, U.S., Cat No. DPC900), and the absorbance of each diluted serum at 450 nm was detected using the full-automatic microplate reader (SYNERGY™ MX, Biotek company) according to the procedures in the instruction of the kit. According to each dilution concentration of the standard in the kit and its corresponding absorbance value, a standard curve was plotted using the linear regression function of Graphpad 5.0 software, and the corresponding linear equation was obtained:  $Y = aX + b$ , wherein  $a$  is the slope of the fitted straight line, and  $b$  is the corresponding  $Y$  value (intercept) when  $X = 0$ . The PCSK9 protein content in the diluted serum can be calculated by bringing the measured absorbance value of each diluted serum into the above equation. The PCSK9 protein content in the serum obtained at each time point can be obtained according to the dilution times.

Normalized PCSK9 protein level = (PCSK9 protein content after administration/PCSK9 protein content before administration)

Inhibition rate against the expression of PCSK9 protein =  $(1 - \text{PCSK9 protein content after administration} / \text{PCSK9 protein content before administration}) \times 100\%$ ;

wherein the PCSK9 protein content before administration is the PCSK9 protein content of D-9.

The PCSK9 protein content at each time point after administration was normalized based on the PCSK9 protein content before administration; the PCSK9 protein level before administration was defined as 100%, and is represented by D0 in Figure 2; after cynomolgus monkey were given a single subcutaneous injection of 9 mg/kg of Conjugate 4, the normalized PCSK9 protein levels in serum at different time points are shown in Figure 2.

(2-2-2) Detection of PCSK9 protein content in the serum of cynomolgus monkeys to which Conjugate 1 or 7 was administered

The content of PCSK9 protein in the serum of cynomolgus monkeys which were given Conjugate 1 or 7 was detected using the same method as in (2-2-1) except that (1) the serum of D-14, D-7, D8, D15, D22, D29, D36, D43, D50, D57, D64 and D85 were selected; and (2) the PCSK9 protein content before administration was the arithmetic mean of the two PCSK9 protein contents of D-14 and D-7. After cynomolgus monkeys were given a single subcutaneous injection of 9mg/kg of Conjugate 1 or 7, the normalized PCSK9 protein levels in serum obtained at different time points are shown in Figure 2.

As can be seen from Figure 2, on day 14 after single administration, Conjugate 4 showed an inhibition rate of more than 90% against the expression of PCSK9 protein in NHP serum, and maintained an inhibition rate of more than 50% against the expression of PCSK9 protein until week 12 after administration.

5

For Conjugate 7, on day 22 after single administration, it showed an inhibition rate of 82% against the expression of PCSK9 protein in NHP serum, and maintained an inhibition rate of more than 66% against the expression of PCSK9 protein until week 9 after administration.

10 For Conjugate 1, on day 15 after single administration, it showed an inhibition rate of 83% against the expression of PCSK9 protein in NHP serum, and maintained an inhibition rate of more than 65% against the expression of PCSK9 protein until week 7 after administration.

(2-3) Detection of blood lipid (LDL-c and CHO) content in serum

15

(2-3-1) Detection of blood lipid (LDL-c and CHO) content in serum of cynomolgus monkeys to which Conjugate 4 was administered

20 The serum obtained at each time point after the administration of Conjugate 4 in (2-2) was selected, and the blood lipid (LDL-c or CHO) content in serum obtained at each time point was measured by a full-automatic biochemical analyzer (Hitachi 7060) using the LDL-C assay kit (DENUO CH7538, Shanghai Zhicheng Biotechnology Co., Ltd.) or the CHOL assay kit (DENUO CH7532, Shanghai Zhicheng Biotechnology Co., Ltd.) according to the procedures in its instruction.

25 The normalized blood lipid level = (the blood lipid content after administration/the blood lipid content before administration)  $\times$  100%.

The inhibition rate against blood lipid = (1 - the blood lipid content after administration/the blood lipid content before administration)  $\times$  100%.

30

Therein, the blood lipid content before administration is the arithmetic mean of the three blood lipid contents of D-15, D-9 and D1 before administration; and blood lipid refers to LDL-c or CHO.

35 The blood lipid content at each time point after administration was normalized based on the blood lipid content before administration; the blood lipid level before administration was defined as 100%, and is represented by D0 in Figure 3 and Figure 4.

40 After cynomolgus monkeys was given a single subcutaneous injection of 9 mg/kg of Conjugate 4, the normalized LDL-c levels and normalized CHO levels in serum at different time points are shown in Figure 3 and Figure 4 respectively.

(2-3-2) Detection of blood lipid (LDL-c and CHO) content in the serum of cynomolgus monkeys to which Conjugate 7 was administered

45 The content of blood lipid (LDL-c and CHO) in the serum of cynomolgus monkeys to which Conjugate 7 was administered was detected using the same method as in (2-3-1) except that (1) the

serum obtained at each time point after the administration of Conjugate 7 in (2-2) was selected for detection of blood lipid; and (2) the content of blood lipid before administration was the arithmetic mean of the three blood lipid contents of D-14, D-7 and D1 before administration. After cynomolgus monkeys were given a single subcutaneous injection of 9mg/kg of Conjugate 7, the normalized LDL-c levels and normalized CHO levels in serum obtained at different time points are shown in Figure 3 and Figure 4 respectively.

As shown in Figure 3, from the 14<sup>th</sup> day after single administration, Conjugate 4 exhibited a significant inhibitory effect on LDL-c. On day 22 after administration, Conjugate 4 showed an inhibition rate of up to 64% against LDL-c. Conjugate 4 maintained an inhibition rate of more than 50% against LDL-c from week 2 to week 11 after administration. The LDL-c level in the serum of NHP to which Conjugate 4 was administered was continuously lower than that before administration over the observation period of 127 days.

As to Conjugate 7, on day 22 after single administration, Conjugate 7 showed an inhibition rate of 36% against LDL-c. Conjugate 7 maintained an inhibition rate of about 30% against LDL-c from week 2 to week 4 after administration. The LDL-c level in the serum of NHP to which Conjugate 7 was administered was continuously lower than that before administration over the observation period of 11 weeks.

As shown in Figure 4, from the 14<sup>th</sup> day after single administration, Conjugate 4 also exhibited a significant inhibitory effect on CHO. On day 22 after administration, Conjugate 4 showed an inhibition rate of up to 38% against CHO. Conjugate 4 maintained an inhibition rate of more than 30% against CHO from week 2 to week 8 after administration. The CHO level in the serum of NHP to which Conjugate 4 was administered was continuously lower than that before administration over the observation period of 127 days.

As to Conjugate 7, on day 22 after single administration, Conjugate 7 showed an inhibition rate of 38% against CHO. Conjugate 7 maintained an inhibition rate of basically more than 30% against CHO from week 2 to week 8 after administration. The CHO level in the serum of NHP to which Conjugate 7 was administered was continuously lower than that before administration over the observation period of 11 weeks.

(2-4) Blood routine and coagulation function tests before and after liver puncture

Before and after the liver puncture, the animals to which Conjugate 4 or 5 was administered were subjected to intravenous blood sampling. Blood routine and coagulation function were detected using the full-automatic five classification hematology analyzer (ADVIA 2120/ADVIA 2120i, Siemens company, Germany) and the full-automatic blood coagulation analyzer (CA-7000/CS-2000i, Sysmex company, Japan) respectively. The results show that administration of each conjugate has no significant effect on the blood routine and coagulation function, as compared with before administration, showing that the siRNA conjugates of the present disclosure have good biological safety.

Experimental example 3 Evaluation of toxicity of siRNA conjugates in rats and mice

In this experimental example, the toxic reactions to the siRNA conjugates of the present disclosure in small animals were evaluated by observing the clinical toxic reactions in rats or mice which were given Conjugate 1, 4 or 7, detecting the liver weight, blood routine, blood biochemistry, blood lipid and other indicators, and performing gross anatomy and histopathological examinations.

5

### (3-1) Evaluation of toxicity in rats

#### (3-1-1) Toxicity test in SD rats to which Conjugate 1 or 4 was administered

- 10 SPF grade SD rats aged 6-9 weeks and weighing 210-250 g (purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., production license number: SCXK (Beijing) 2016-0011 or SCXK (Beijing) 2016-0006), all of which were males, were divided into 3 groups according to their body weights, with 5 rats in each group. The rats were subcutaneously injected with Conjugate 1, Conjugate 4 or a 1×PBS (pH 7.4) control separately. The dosage of the conjugate was calculated  
15 based on body weight. Each conjugate was prepared with 1×PBS (pH 7.4) into a 60 mg/ml solution, the administration volume was 5 ml/kg, and the administration dose was 300 mg/kg.

After single subcutaneous administration, the following indicators were detected:

- 20 (1) Observation of general status: observation of general status was performed twice per day;
- (2) Body weight: body weight was measured on D1 before administration, D3, D6, D10 and D14 after administration, and D15 (when dissection was performed);
- 25 (3) Food intake: food intake was measured on D2-3, D6-7 and D12-13 respectively;
- (4) Hematology: On D15 before dissection, 1.9 ml blood was collected from the abdominal aorta of the rat, wherein 1.0 ml blood sample was anticoagulated with EDTA-K2, and the whole blood was directly injected to detect the following blood cytological indicators: red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), hemoglobin (HGB),  
30 hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte count (RET), the percentage of reticulocytes (RET%), the count and percentage of neutrophils (NEU), the count and percentage of lymphocytes (LYM), the count and percentage of monocytes (MONO), the count and percentage of eosinophils (EOS), and the count and percentage of  
35 basophils (BASO); and the remaining 0.9 ml blood sample was anticoagulated with sodium citrate and centrifuged at 1800×g at 15°C to 25°C for 10 minutes to separate plasma used for detecting blood coagulation time (prothrombin time (PT) and activated partial thrombin time (APTT));
- 40 (5) Blood biochemistry: after administration, 0.6 ml of blood was collected from the jugular vein of the rat on D2, and 3 ml of blood was collected from the abdominal aorta of the rat on D15 before dissection; the blood samples, none of which was anticoagulated, were centrifuged at 1800×g at 15°C to 25°C for 10 minutes and serum was separated for  
45 measuring blood biochemical indicators of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH),  $\gamma$ -glutamyl transpeptidase (GGT), urea (Urea), creatinine (Crea),

sodium ion concentration ( $\text{Na}^+$ ), potassium ion concentration ( $\text{K}^+$ ), chloride ion ( $\text{Cl}^-$ ), blood glucose (GLU), total bilirubin (TBIL), total protein (TP), albumin (ALB) and albumin/globulin ratio (A/G).

- 5 (6) Gross anatomy and histopathological examination: animals were dissected on D15, each organ was weighed, and histopathological examination was performed.

#### (3-1-2) Toxicity test in SD rats to which Conjugate 7 was administered

- 10 The toxicity in SD rats to which Conjugate 7 was administered was detected using the same method as in (3-1-1) except that Conjugate 4 was replaced by Conjugate 7; Conjugate 7 was prepared with 1×PBS (pH 7.4) into a 20 mg/ml solution and a 6 mg/ml solution respectively; the administration volume was 5 ml/kg, and the administration dose was 100 mg/kg and 30 mg/kg respectively. For indicator (5) of blood biochemistry, blood was collected on D8 and D15 respectively before  
15 dissection after administration.

The results show that none of the conjugates showed obvious toxicity in rats.

#### (3-2) Evaluation of toxicity in mice

- 20 Each group of 10 SPF grade ICR mice aged 5-6 weeks (purchased from SPF (Beijing) biotechnology co., LTD.), half of which were males, were injected with Conjugate 1, Conjugate 4 or Conjugate 7 subcutaneously. The administration was performed once a week for 2 consecutive weeks, 3 times in total (on D1, D8 and D15 respectively). The dosage of the conjugate was  
25 calculated based on body weight. Each conjugate was prepared with 1×PBS (pH 7.4) into a 30 mg/ml solution, the administration volume was 10 ml/kg, and the administration dose was 300 mg/kg.

After administration was performed 3 times, the following indicators were detected:

- 30 (1) Observation of general status: observation of general status was performed once per day;
- (2) Body weight: body weight was measured on D1, D8 and D15, and before administration and D16 (when the dissection was performed) respectively;  
35 (3) Food intake: food intake was measured every week;
- (4) Blood biochemistry: On D16 when dissection was performed, 1 ml of blood was collected by eyeball removal, and was not anticoagulated; the blood was first incubated at 37°C for  
40 60 min, and then centrifuged at 4°C at 3000 rpm for 15 min to obtain serum for blood biochemical measurement; the test indicators were the same as the blood biochemical indicators in the rat toxicity test.
- (6) Gross anatomy and histopathological examination: animals were dissected on D16, each  
45 organ was weighed, and histopathological examination was performed.

The results show that none of the conjugates showed obvious toxicity in mice.

The above results show that the conjugates of the present disclosure have good biological safety.

5 Some embodiments of the present disclosure are described in detail above, but the present disclosure is not limited to the specific details of the above embodiments. Various simple variations to the technical solutions of the present disclosure can be made within the scope of the technical concept of the present disclosure, and these simple variations are also within the scope of the present disclosure.

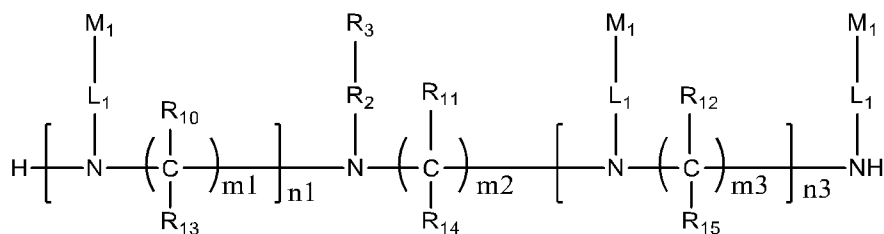
10 It is to be noted that each of the specific technical features described in the above embodiments can be combined in any suitable manner provided that no contradiction is caused. In order to avoid unnecessary repetition, various possible combination manners are no longer described in the present disclosure.

15 In addition, various different embodiments of the present disclosure may also be combined as long as it does not deviate from the idea of the present disclosure, which should also be regarded as the disclosure of the present disclosure.

20

## CLAIMS

1. An siRNA conjugate having a structure as shown by Formula (308):



Formula (308),

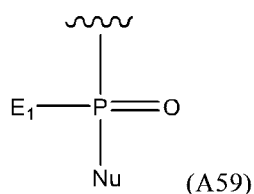
wherein

n1 is an integer of 1-3, and n3 is an integer of 0-4;

each m1, m2, or m3 independently of one another is an integer of 2-10;

R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub>, R<sub>13</sub>, R<sub>14</sub>, or R<sub>15</sub> independently of one another is H, or selected from the group consisting of C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>1</sub>-C<sub>10</sub> haloalkyl, and C<sub>1</sub>-C<sub>10</sub> alkoxy;

R<sub>3</sub> is a group having a structure as shown by Formula (A59):



wherein,

E<sub>1</sub> is OH, SH or BH<sub>2</sub>;

Nu is an siRNA;

the siRNA comprises a sense strand and an antisense strand; each nucleotide in the siRNA is independently a modified or unmodified nucleotide; wherein the sense strand comprises a nucleotide sequence I, and the antisense strand comprises a nucleotide sequence II; the nucleotide sequence I and the nucleotide sequence II are at least partly reverse complementary to form a double-stranded region; the nucleotide sequence I and the nucleotide sequence II are the sequences selected from one of the following groups i)-vi):

i) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 1 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 2 have an equal length and no more than 3 nucleotide differences:

5'- AAGCAAGCAGACAUUUAUZ<sub>1</sub> -3' (SEQ ID NO: 1);

5'- Z<sub>2</sub>AUAAAUGUCUGCUUGCUU -3' (SEQ ID NO: 2),

wherein, Z<sub>1</sub> is C and Z<sub>2</sub> is G, and

the nucleotide sequence I comprises a nucleotide Z<sub>3</sub> at the position corresponding to Z<sub>1</sub>; the nucleotide sequence II comprises a nucleotide Z<sub>4</sub> at the position corresponding to Z<sub>2</sub>, wherein Z<sub>4</sub> is the first nucleotide at 5' terminal of the antisense strand;

ii) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 61 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 62 have an equal length and no more than 3 nucleotide differences:

5        5'- UUUGUAGCAUUUUUAUUAZ<sub>5</sub> -3' (SEQ ID NO: 61);  
        5'- Z<sub>6</sub>UAAUAAAAAUGCUACAAA -3' (SEQ ID NO: 62),

wherein, Z<sub>5</sub> is A and Z<sub>6</sub> is U, and

the nucleotide sequence I comprises a nucleotide Z<sub>7</sub> at the position corresponding to Z<sub>5</sub>; the nucleotide sequence  
 10       II comprises a nucleotide Z<sub>8</sub> at the position corresponding to Z<sub>6</sub>, wherein Z<sub>8</sub> is the first nucleotide at 5' terminal  
        of the antisense strand;

iii) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 121 have an equal length  
 and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown  
 15       by SEQ ID NO: 122 have an equal length and no more than 3 nucleotide differences:

       5'- GCCUGGAGUUUAUUCGGAZ<sub>9</sub> -3' (SEQ ID NO: 121);  
        5'- Z<sub>10</sub>UCCGAAUAAACUCCAGGC -3' (SEQ ID NO: 122),

20       wherein, Z<sub>9</sub> is A and Z<sub>10</sub> is U, and

the nucleotide sequence I comprises a nucleotide Z<sub>11</sub> at the position corresponding to Z<sub>9</sub>; the nucleotide  
 sequence II comprises a nucleotide Z<sub>12</sub> at the position corresponding to Z<sub>10</sub>, wherein Z<sub>12</sub> is the first nucleotide  
 at 5' terminal of the antisense strand;

iv) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 181 have an equal length  
 and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown  
 25       by SEQ ID NO: 182 have an equal length and no more than 3 nucleotide differences:

       5'- CUGUUUUGCUUUUGUAACZ<sub>13</sub> -3' (SEQ ID NO: 181);  
 30       5'- Z<sub>14</sub>GUUACAAAAGCAAAACAG -3' (SEQ ID NO: 182),

wherein, Z<sub>13</sub> is U and Z<sub>14</sub> is A, and

the nucleotide sequence I comprises a nucleotide Z<sub>15</sub> at the position corresponding to Z<sub>13</sub>; the nucleotide  
 sequence II comprises a nucleotide Z<sub>16</sub> at the position corresponding to Z<sub>14</sub>, wherein Z<sub>16</sub> is the first nucleotide  
 35       at 5' terminal of the antisense strand;

v) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 241 have an equal length  
 and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown  
 by SEQ ID NO: 242 have an equal length and no more than 3 nucleotide differences:

40       5'- GGUUUUGUAGCAUUUUUAZ<sub>17</sub> -3' (SEQ ID NO: 241);  
        5'- Z<sub>18</sub>UAAAAAUGCUACAAAACC -3' (SEQ ID NO: 242),

wherein, Z<sub>17</sub> is U and Z<sub>18</sub> is A, and

the nucleotide sequence I comprises a nucleotide Z<sub>19</sub> at the position corresponding to Z<sub>17</sub>; the nucleotide  
 45       sequence II comprises a nucleotide Z<sub>20</sub> at the position corresponding to Z<sub>18</sub>, wherein Z<sub>20</sub> is the first nucleotide  
        at 5' terminal of the antisense strand; and

vi) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 301 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 302 have an equal length and no more than 3 nucleotide differences:

5

5'-GUGACUUUUUAAAAUAAAZ<sub>21</sub>-3' (SEQ ID NO: 301);

5'-Z<sub>22</sub>UUUAUUUUAAAAAGUCAC-3' (SEQ ID NO: 302),

wherein, Z<sub>21</sub> is A and Z<sub>22</sub> is U, and

10 the nucleotide sequence I comprises a nucleotide Z<sub>23</sub> at the position corresponding to Z<sub>21</sub>; the nucleotide sequence II comprises a nucleotide Z<sub>24</sub> at the position corresponding to Z<sub>22</sub>, wherein Z<sub>24</sub> is the first nucleotide at 5' terminal of the antisense strand;

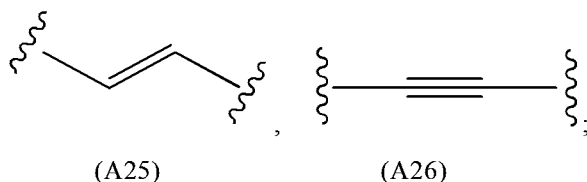
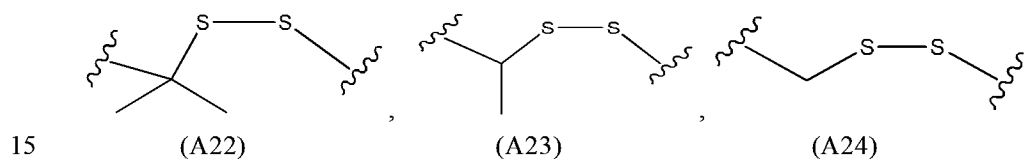
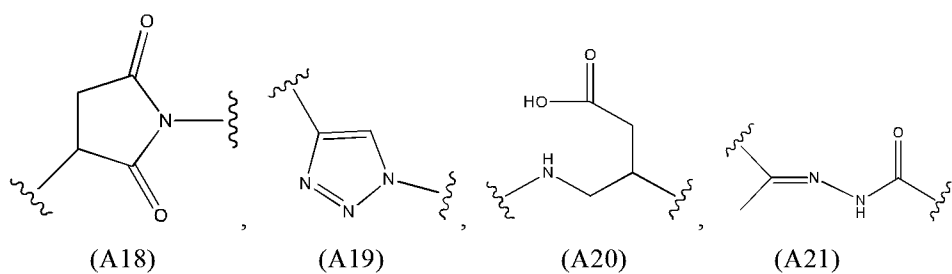
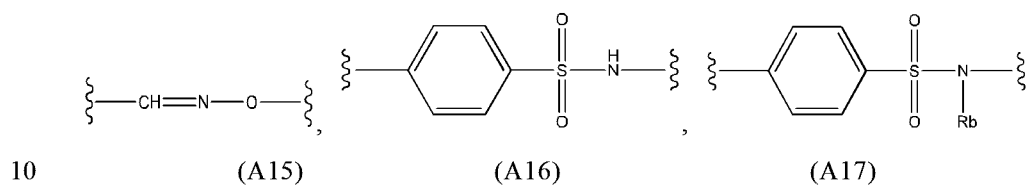
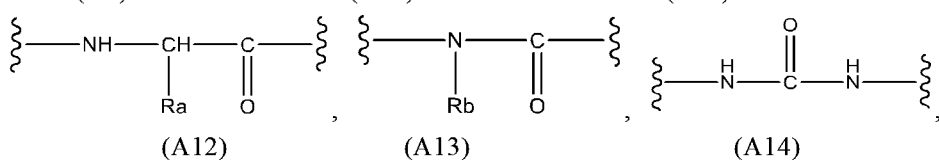
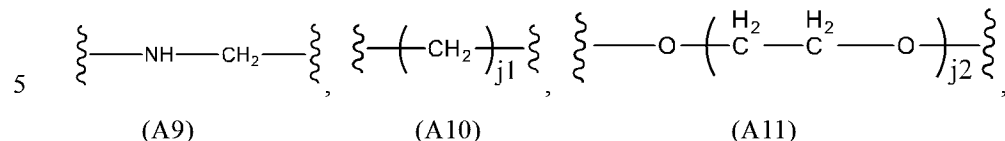
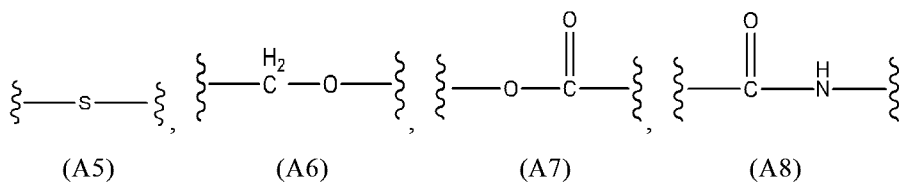
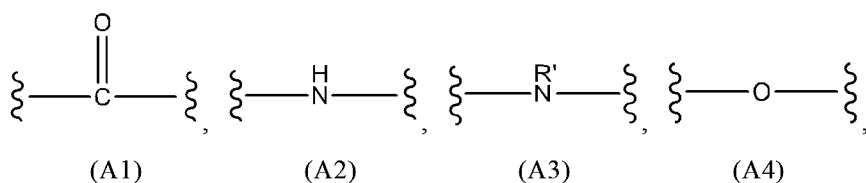
15 R<sub>2</sub> is a linear alkylene of 1 to 20 carbon atoms in length, wherein one or more carbon atoms are optionally replaced with any one or more groups selected from the group consisting of: C(O), NH, O, S, CH=N, S(O)<sub>2</sub>, C<sub>2</sub>-C<sub>10</sub> alkenylene, C<sub>2</sub>-C<sub>10</sub> alkynylene, C<sub>6</sub>-C<sub>10</sub> arylene, C<sub>3</sub>-C<sub>18</sub> heterocyclylene, and C<sub>5</sub>-C<sub>10</sub> heteroarylene; and wherein R<sub>2</sub> optionally has any one or more substituents selected from the group consisting of: C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>6</sub>-C<sub>10</sub> aryl, C<sub>5</sub>-C<sub>10</sub> heteroaryl, C<sub>1</sub>-C<sub>10</sub> haloalkyl, -OC<sub>1</sub>-C<sub>10</sub> alkyl, -OC<sub>1</sub>-C<sub>10</sub> alkylphenyl, -C<sub>1</sub>-C<sub>10</sub> alkyl-OH, -OC<sub>1</sub>-C<sub>10</sub> haloalkyl, -SC<sub>1</sub>-C<sub>10</sub> alkyl, -SC<sub>1</sub>-C<sub>10</sub> alkylphenyl, -C<sub>1</sub>-C<sub>10</sub> alkyl-SH, -SC<sub>1</sub>-C<sub>10</sub> haloalkyl, halo, -OH, -SH, 20 -NH<sub>2</sub>, -C<sub>1</sub>-C<sub>10</sub> alkyl-NH<sub>2</sub>, -N(C<sub>1</sub>-C<sub>10</sub> alkyl)(C<sub>1</sub>-C<sub>10</sub> alkyl), -NH(C<sub>1</sub>-C<sub>10</sub> alkyl), -N(C<sub>1</sub>-C<sub>10</sub> alkyl) (C<sub>1</sub>-C<sub>10</sub> alkylphenyl), -NH(C<sub>1</sub>-C<sub>10</sub> alkylphenyl), cyano, nitro, -CO<sub>2</sub>H, -C(O)O(C<sub>1</sub>-C<sub>10</sub> alkyl), -CON(C<sub>1</sub>-C<sub>10</sub> alkyl)(C<sub>1</sub>-C<sub>10</sub> alkyl), -CONH(C<sub>1</sub>-C<sub>10</sub> alkyl), -CONH<sub>2</sub>, -NHC(O)(C<sub>1</sub>-C<sub>10</sub> alkyl), -NHC(O)(phenyl), -N(C<sub>1</sub>-C<sub>10</sub> alkyl)C(O)(C<sub>1</sub>-C<sub>10</sub> alkyl), -N(C<sub>1</sub>-C<sub>10</sub> alkyl)C(O)(phenyl), -C(O)C<sub>1</sub>-C<sub>10</sub> alkyl, -C(O)C<sub>1</sub>-C<sub>10</sub> alkylphenyl, -C(O)C<sub>1</sub>-C<sub>10</sub> haloalkyl, -OC(O)C<sub>1</sub>-C<sub>10</sub> alkyl, -SO<sub>2</sub>(C<sub>1</sub>-C<sub>10</sub> alkyl), -SO<sub>2</sub>(phenyl), -SO<sub>2</sub>(C<sub>1</sub>-C<sub>10</sub> haloalkyl), - 25 SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NH(C<sub>1</sub>-C<sub>10</sub> alkyl), -SO<sub>2</sub>NH(phenyl), -NHSO<sub>2</sub>(C<sub>1</sub>-C<sub>10</sub> alkyl), -NHSO<sub>2</sub>(phenyl), and -NHSO<sub>2</sub>(C<sub>1</sub>-C<sub>10</sub> haloalkyl);

each L<sub>1</sub> independently is a linear alkylene of 1 to 70 carbon atoms in length, wherein one or more carbon atoms are optionally replaced with any one or more groups selected from the group consisting of: C(O), NH, O, S, 30 CH=N, S(O)<sub>2</sub>, C<sub>2</sub>-C<sub>10</sub> alkenylene, C<sub>2</sub>-C<sub>10</sub> alkynylene, C<sub>6</sub>-C<sub>10</sub> arylene, C<sub>3</sub>-C<sub>18</sub> heterocyclylene, and C<sub>5</sub>-C<sub>10</sub> heteroarylene; and wherein L<sub>1</sub> optionally has any one or more substituents selected from the group consisting of: C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>6</sub>-C<sub>10</sub> aryl, C<sub>5</sub>-C<sub>10</sub> heteroaryl, C<sub>1</sub>-C<sub>10</sub> haloalkyl, -OC<sub>1</sub>-C<sub>10</sub> alkyl, -OC<sub>1</sub>-C<sub>10</sub> alkylphenyl, -C<sub>1</sub>-C<sub>10</sub> alkyl-OH, -OC<sub>1</sub>-C<sub>10</sub> haloalkyl, -SC<sub>1</sub>-C<sub>10</sub> alkyl, -SC<sub>1</sub>-C<sub>10</sub> alkylphenyl, -C<sub>1</sub>-C<sub>10</sub> alkyl-SH, -SC<sub>1</sub>-C<sub>10</sub> haloalkyl, halo, -OH, -SH, -NH<sub>2</sub>, -C<sub>1</sub>-C<sub>10</sub> alkyl-NH<sub>2</sub>, -N(C<sub>1</sub>-C<sub>10</sub> alkyl)(C<sub>1</sub>-C<sub>10</sub> alkyl), -NH(C<sub>1</sub>-C<sub>10</sub> alkyl), -N(C<sub>1</sub>-C<sub>10</sub> 35 alkyl)(C<sub>1</sub>-C<sub>10</sub> alkylphenyl), -NH(C<sub>1</sub>-C<sub>10</sub> alkylphenyl), cyano, nitro, -CO<sub>2</sub>H, -C(O)O(C<sub>1</sub>-C<sub>10</sub> alkyl), -CON(C<sub>1</sub>-C<sub>10</sub> alkyl)(C<sub>1</sub>-C<sub>10</sub> alkyl), -CONH(C<sub>1</sub>-C<sub>10</sub> alkyl), -CONH<sub>2</sub>, -NHC(O)(C<sub>1</sub>-C<sub>10</sub> alkyl), -NHC(O)(phenyl), -N(C<sub>1</sub>-C<sub>10</sub> alkyl)C(O)(C<sub>1</sub>-C<sub>10</sub> alkyl), -N(C<sub>1</sub>-C<sub>10</sub> alkyl)C(O)(phenyl), -C(O)C<sub>1</sub>-C<sub>10</sub> alkyl, -C(O)C<sub>1</sub>-C<sub>10</sub> alkylphenyl, -C(O)C<sub>1</sub>-C<sub>10</sub> haloalkyl, -OC(O)C<sub>1</sub>-C<sub>10</sub> alkyl, -SO<sub>2</sub>(C<sub>1</sub>-C<sub>10</sub> alkyl), -SO<sub>2</sub>(phenyl), -SO<sub>2</sub>(C<sub>1</sub>-C<sub>10</sub> haloalkyl), - SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NH(C<sub>1</sub>-C<sub>10</sub> alkyl), -SO<sub>2</sub>NH(phenyl), -NHSO<sub>2</sub>(C<sub>1</sub>-C<sub>10</sub> alkyl), -NHSO<sub>2</sub>(phenyl), and -NHSO<sub>2</sub>(C<sub>1</sub>- 40 C<sub>10</sub> haloalkyl);

~~~~~ represents the site where a group is covalently linked; and M<sub>1</sub> represents a targeting group.

2. The siRNA conjugate according to claim 1, wherein each L₁ is independently selected from the group consisting of the groups of Formulae (A1)-(A26) and any combination thereof:

45

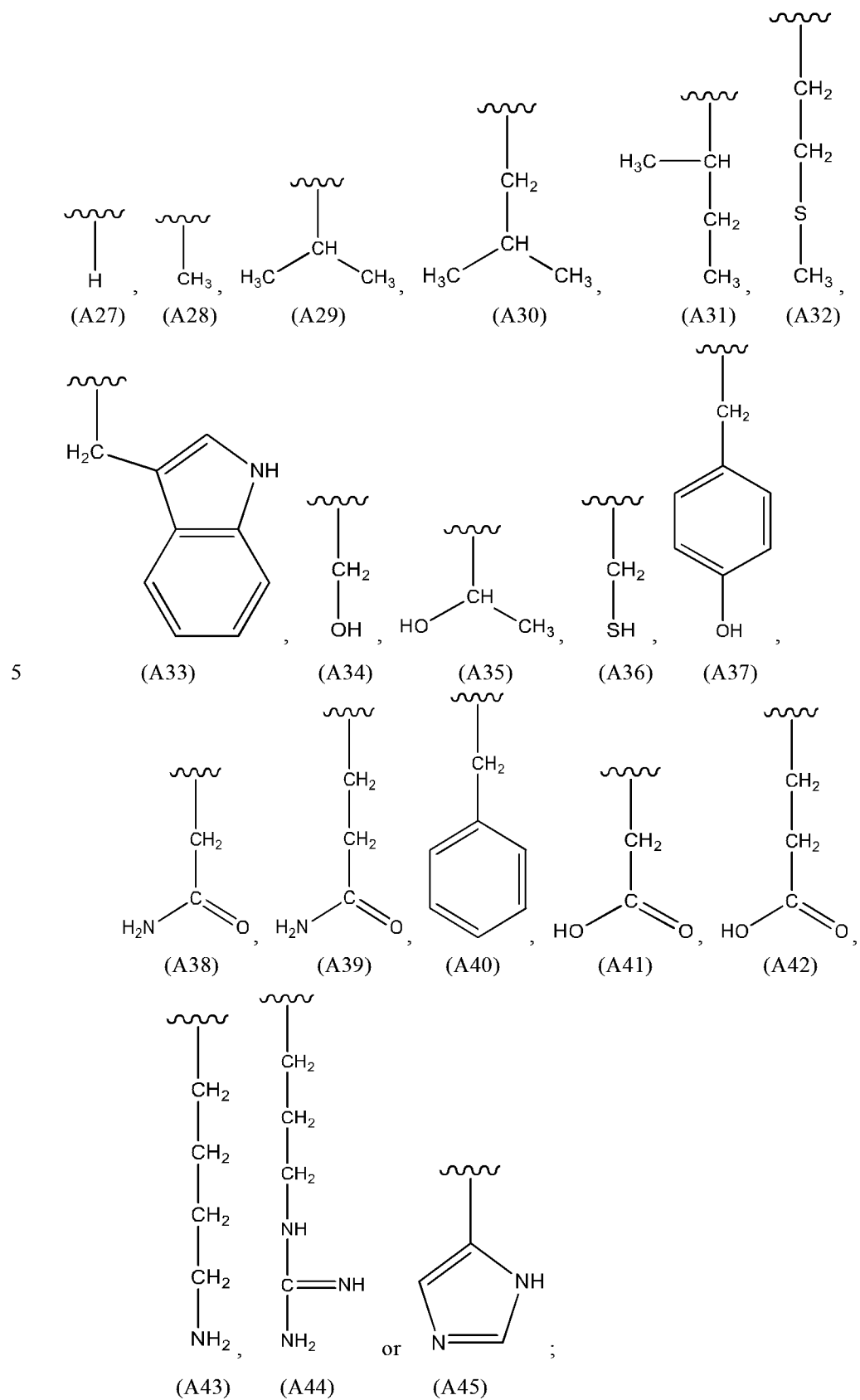


wherein,

each $j1$ is independently an integer of 1-20; each $j2$ is independently an integer of 1-20;

each R' is independently a $\text{C}_1\text{-C}_{10}$ alkyl;

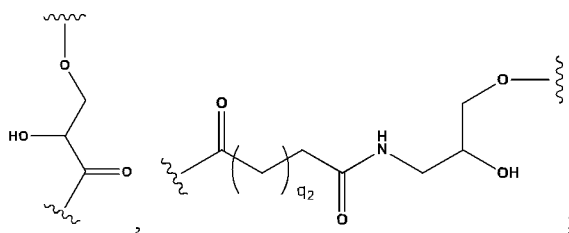
each Ra is independently selected from the group consisting of the groups of Formulae (A27)-(A45) or any combination thereof:



each Rb is independently a C₁-C₁₀ alkyl;

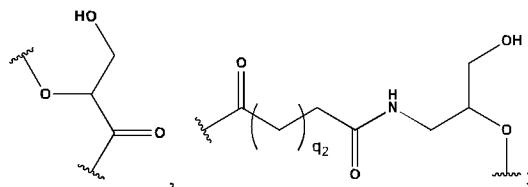
- or L₁ is selected from the connection combinations of one or more of Formulae (A1), (A4), (A5), (A6), (A8), (A10), (A11), and (A13);
- or L₁ is selected from the connection combinations of at least two of Formulae (A1), (A4), (A8), (A10), and (A11);
- 5 or L₁ is selected from the connection combinations of at least two of Formulae (A1), (A8) and (A10);
- or L₁ has a length of 3 to 25 atoms;
- or L₁ has a length of 4 to 15 atoms;
- ~~~~~ represents the site where a group is covalently linked.
- 10 3. The siRNA conjugate according to claim 2, wherein j₁ is an integer of 2-10; j₂ is an integer of 2-10; R' is a C₁-C₄ alkyl; Ra is one of A27, A28, A29, A30, and A31; and Rb is a C₁-C₅ alkyl; or
- j₁ is an integer of 3-5; j₂ is an integer of 3-5; R' is one of methyl, ethyl, and isopropyl; Ra is a group as shown by Formula A27 or A28; and Rb is one of methyl, ethyl, isopropyl, and butyl.
- 15 4. The siRNA conjugate according to any one of claims 1-3, wherein n₁ is an integer of 1-2; n₃ is an integer of 0-1; and n₁+n₃ = 2-3.
5. The siRNA conjugate according to any one of claims 1-4, wherein each m₁, m₂ or m₃ independently of one
- 20 another is an integer of 2-5, and/or m₁ = m₂ = m₃.
6. The siRNA conjugate according to any one of claims 1-5, wherein each of the targeting groups is independently a ligand that has affinity to the asialoglycoprotein receptor on the surface of mammalian hepatocyte; or
- 25 each of the targeting groups is independently an asialoglycoprotein or a saccharide; or
- each of the targeting groups is independently selected from the group consisting of D-mannopyranose, L-mannopyranose, D-arabinose, D-xylofuranose, L-xylofuranose, D-glucose, L-glucose, D-galactose, L-galactose, α-D-mannofuranose, β-D-mannofuranose, α-D-mannopyranose, β-D-mannopyranose, α-D-glucopyranose, β-D-glucopyranose, α-D-glucofuranose, β-D-glucofuranose, α-D-fructofuranose, α-D-fructopyranose, α-D-galactopyranose, β-D-galactopyranose, α-D-galactofuranose, β-D-galactofuranose,
- 30 glucosamine, sialic acid, galactosamine, N-acetylgalactosamine, N-trifluoroacetylgalactosamine, N-propionylgalactosamine, N-n-butyrylgalactosamine, N-isobutyrylgalactosamine, 2-amino-3-O-[(R)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose, 2-deoxy-2-methylamino-L-glucopyranose, 4,6-dideoxy-4-formamido-2,3-di-O-methyl-D-mannopyranose, 2-deoxy-2-sulfoamino-D-glucopyranose, N-glycolyl-α-neuraminic acid, 5-thio-β-D-glucopyranose, methyl 2,3,4-tris-O-acetyl-1-thio-6-O-trityl-α-D-glucopyranoside,
- 35 4-thio-β-D-galactopyranose, ethyl 3,4,6,7-tetra-O-acetyl-2-deoxy-1,5-dithio-α-D-glucuheptopyranoside, 2,5-anhydro-D-allonitrile, ribose, D-ribose, D-4-thioribose, L-ribose, L-4-thioribose; or
- at least one or each of the targeting groups is galactose or N-acetylgalactosamine.
- 40 7. The siRNA conjugate according to any one of claims 1-6, wherein R₁₀, R₁₁, R₁₂, R₁₃, R₁₄, or R₁₅ independently of one another is H, methyl or ethyl; or
- each of R₁₀, R₁₁, R₁₂, R₁₃, R₁₄, and R₁₅ is H.
8. The siRNA conjugate according to any one of claims 1-7, wherein R₂ comprises both a site linking to the N
- 45 atom on the nitrogenous backbone and a site linking to the P atom in R₃; or
- in R₂, the site linking to the N atom on the nitrogenous backbone forms an amide bond with the N atom, and

the site linking to the P atom in R₃ forms a phosphoester bond with the P atom;
or R₂ is a group having a structure as shown by any one of Formula (B5), (B6), (B5') or (B6'),



(B5)

(B6)

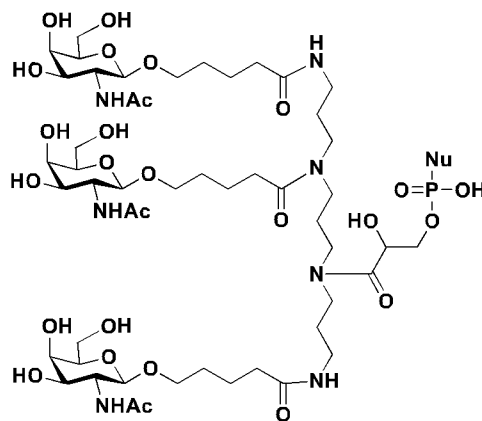


(B5')

(B6')

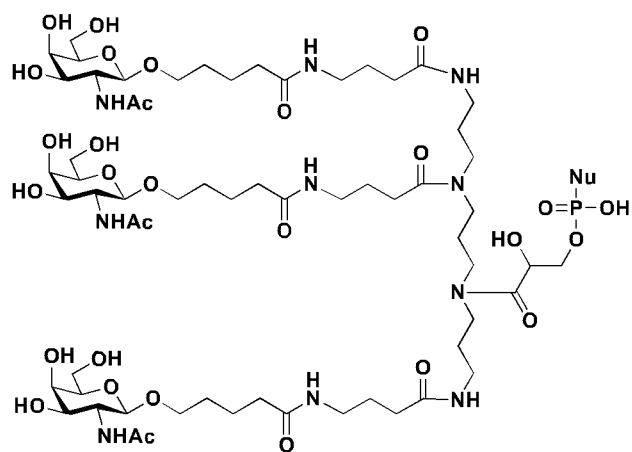
wherein represents the site where the group is covalently linked; and q₂ is an integer of 1-10.

- 10 9. The siRNA conjugate according to any one of claims 1-8, wherein the conjugate has a structure as shown by Formula (403), (404), (405), (406), (407), (408), (409), (410), (411), (412), (413), (414), (415), (416), (417), (418), (419), (420), (421), or (422):

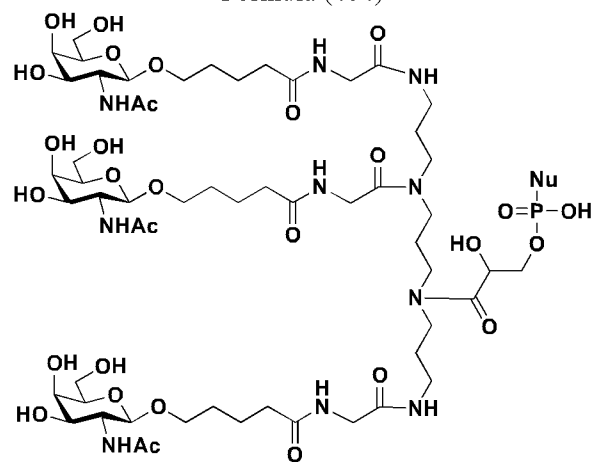


Formula (403)

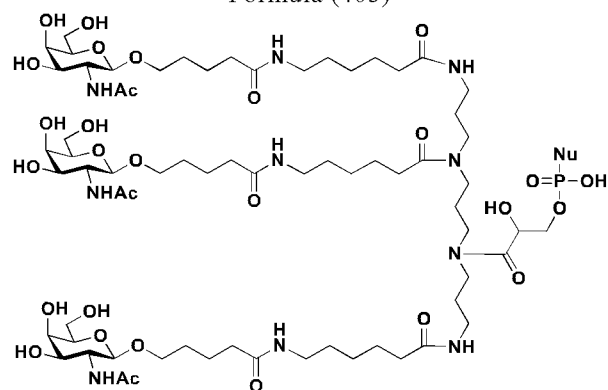
15



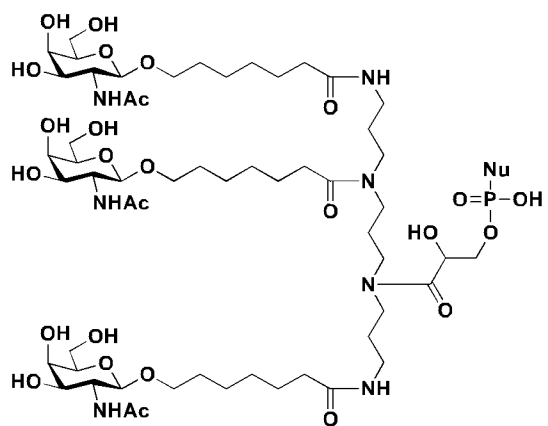
Formula (404)



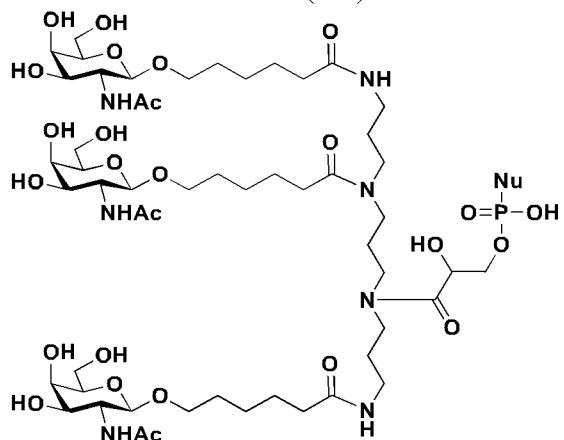
Formula (405)



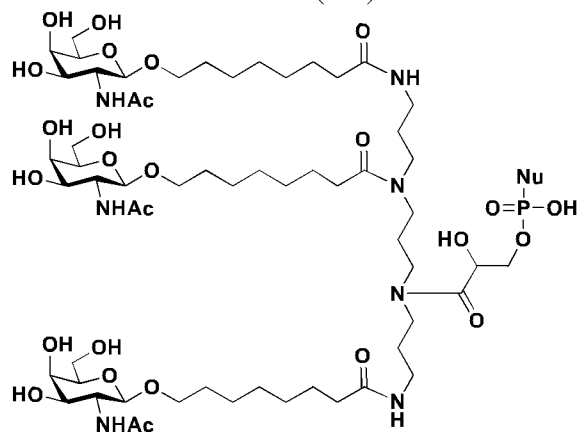
Formula (406)



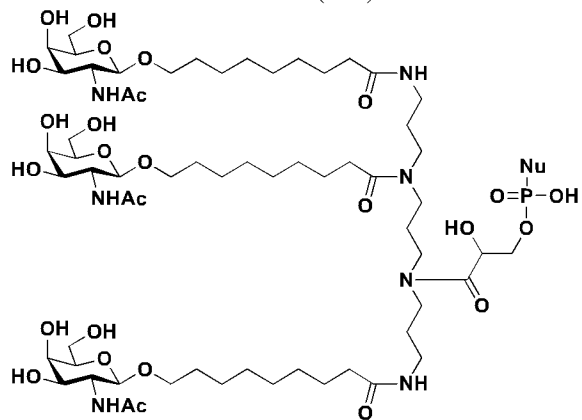
Formula (407)



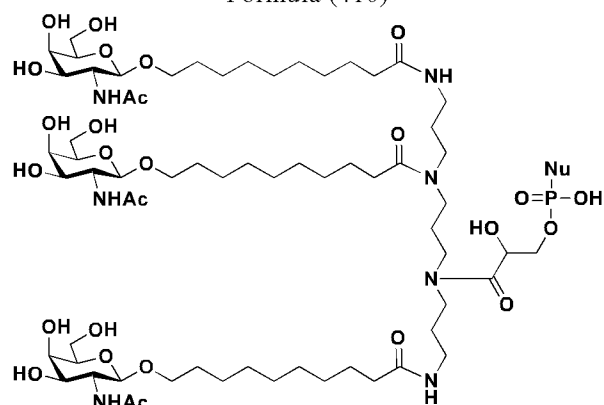
Formula (408)



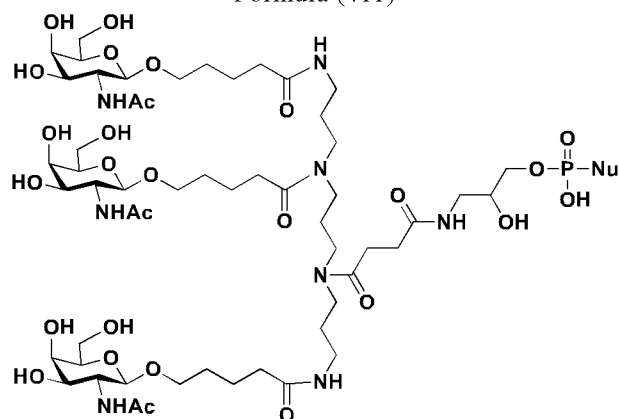
Formula (409)



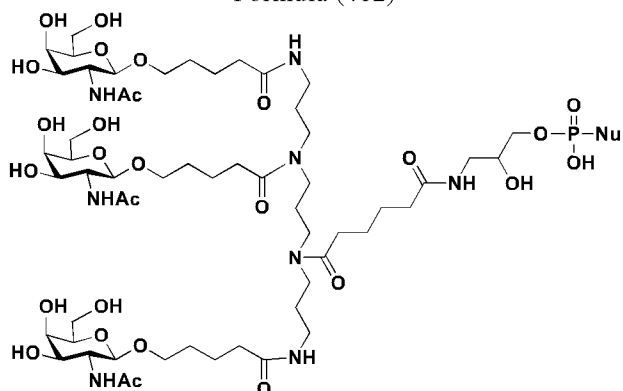
Formula (410)



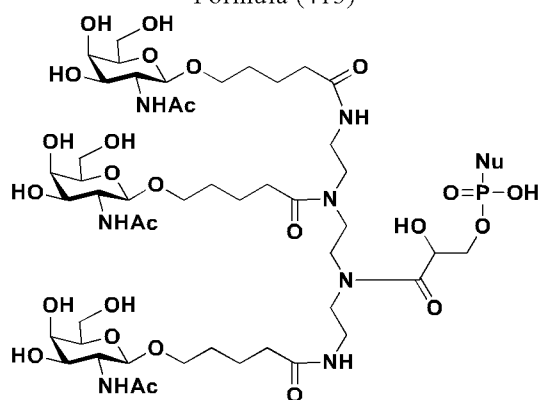
Formula (411)



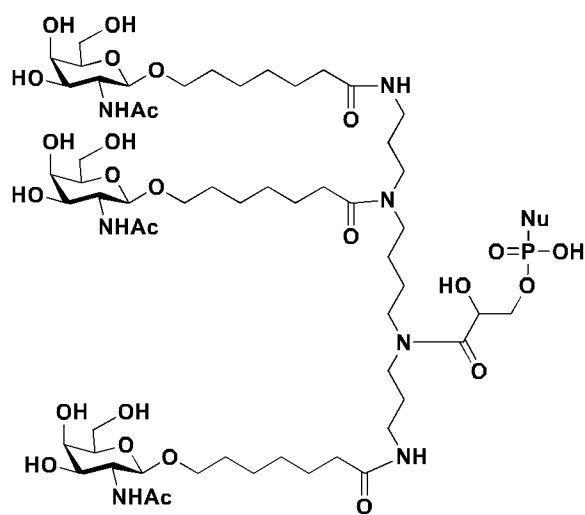
Formula (412)



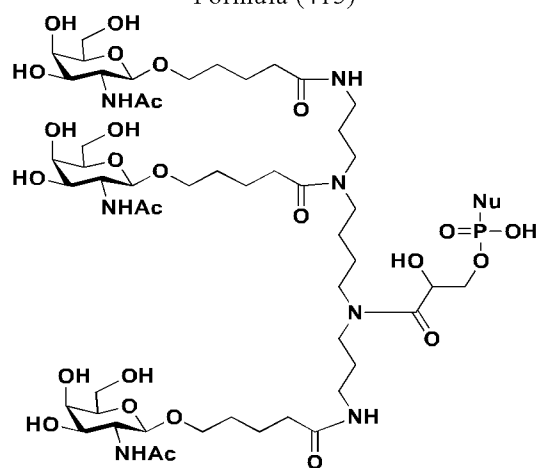
Formula (413)



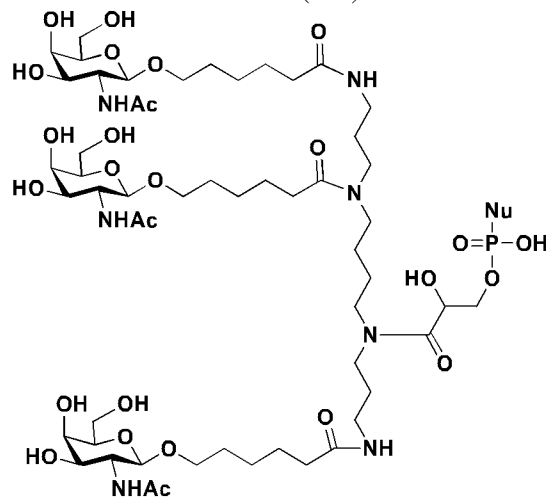
Formula (414)



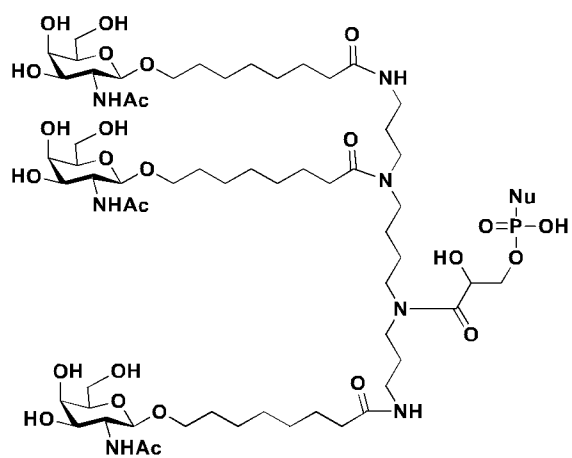
Formula (415)



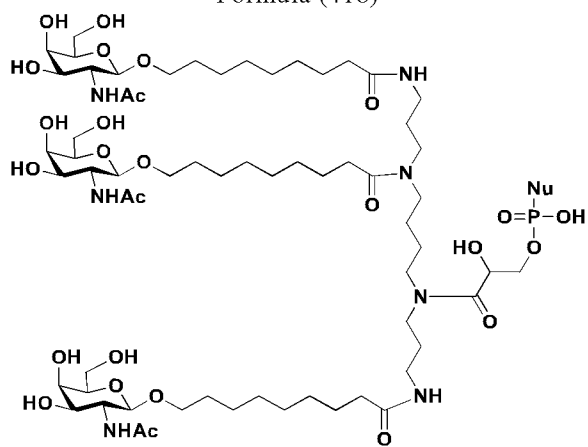
Formula (416)



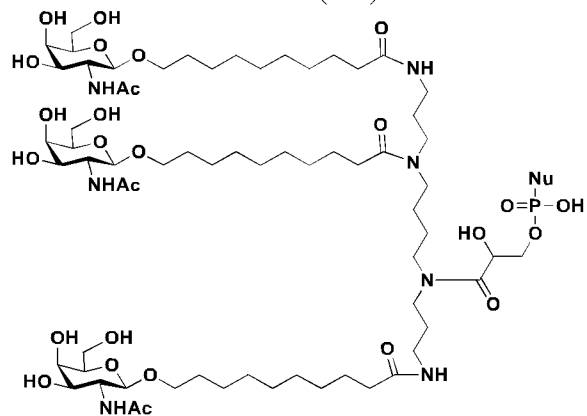
Formula (417)



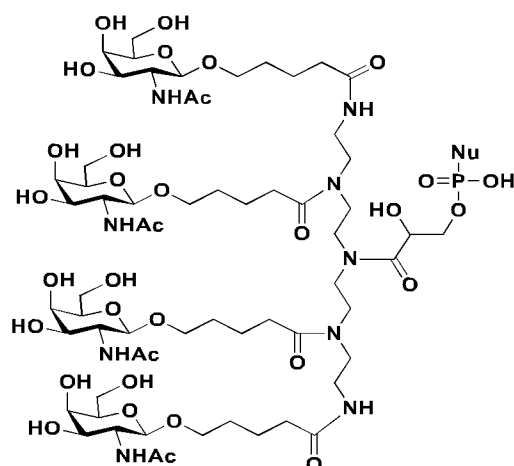
Formula (418)



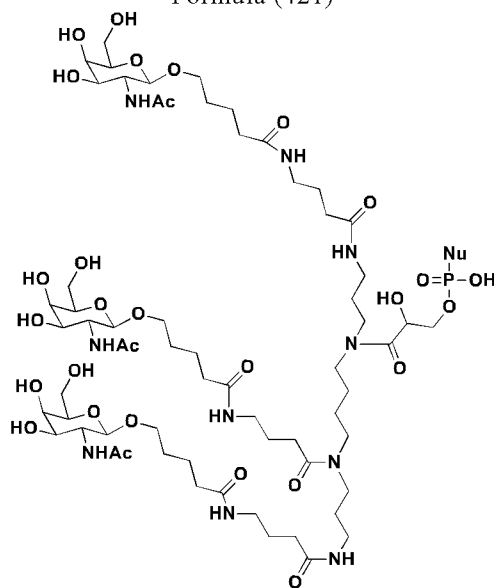
Formula (419)



Formula (420)



Formula (421)



Formula (422).

5

10. The siRNA conjugate according to any one of claims 1-9, wherein the P atom in Formula (A59) is linked to a terminal region of the sense or antisense strand of the siRNA, and the terminal region refers to the first 4 nucleotides counted from one terminal of the sense or antisense strand; or

the P atom in Formula (A59) is linked to one terminal of the sense or antisense strand of the siRNA; or

10 the P atom in Formula (A59) is linked to 3' terminal of the sense strand of the siRNA; or

the P atom in Formula (A59) is linked to position 2', 3', or 5' of a nucleotide in the siRNA by a phosphodiester bond.

11. The siRNA conjugate according to claim 1, wherein i) the nucleotide sequence I has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 1, and/or the nucleotide sequence II has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 2; or

ii) the nucleotide sequence I has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 61, and/or the nucleotide sequence II has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 62; or

iii) the nucleotide sequence I has no more than 1 nucleotide difference from the nucleotide sequence as shown

by SEQ ID NO: 121, and/or the nucleotide sequence II has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 122; or

iv) the nucleotide sequence I has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 181, and/or the nucleotide sequence II has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 182;

v) the nucleotide sequence I has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 241, and/or the nucleotide sequence II has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 242; or

vi) the nucleotide sequence I has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 301, and/or the nucleotide sequence II has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 302.

12. The siRNA conjugate according to claim 1 or 11, wherein i) the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 2 includes a difference at the position Z_4 , wherein Z_4 is selected from A, U or C; or

ii) the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 62 includes a difference at the position Z_8 , wherein Z_8 is selected from A, C or G; or

iii) the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 122 includes a difference at the position Z_{12} , wherein Z_{12} is selected from A, C or G; or

iv) the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 182 includes a difference at the position Z_{16} , wherein Z_{16} is selected from U, C or G;

v) the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 242 includes a difference at the position Z_{20} , wherein Z_{20} is selected from U, C or G; or

vi) the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 302 includes a difference at the position Z_{24} , wherein Z_{24} is selected from A, C or G.

13. The siRNA conjugate according to claim 12, wherein Z_3 is a nucleotide complementary to Z_4 ; or Z_7 is a nucleotide complementary to Z_8 ; or Z_{11} is a nucleotide complementary to Z_{12} ; or Z_{15} is a nucleotide complementary to Z_{16} ; or Z_{19} is a nucleotide complementary to Z_{20} ; or Z_{23} is a nucleotide complementary to Z_{24} .

14. The siRNA conjugate according to any one of claims 1-13, wherein the nucleotide sequence I and the nucleotide sequence II are basically reverse complementary, substantially reverse complementary, or completely reverse complementary to each other; the “basically reverse complementary” means that there is no more than 3 base mispairings between two nucleotide sequences; the “substantially reverse complementary” means that there is no more than 1 base mispairing between two nucleotide sequences; and the “completely

reverse complementary” means that there is no mispairing between two nucleotide sequences.

15. The siRNA conjugate according to any one of claims 11-14, wherein the sense strand further comprises a nucleotide sequence III, the antisense strand further comprises a nucleotide sequence IV; and the nucleotide sequence III and the nucleotide sequence IV independently of one another have a length of 1 to 4 nucleotides; the nucleotide sequence III is linked to 5' terminal of the nucleotide sequence I, the nucleotide sequence IV is linked to 3' terminal of the nucleotide sequence II; the nucleotide sequence III and the nucleotide sequence IV have an equal length, and are substantially reverse complementary or completely reverse complementary to each other; the “substantially reverse complementary” means that there is no more than 1 base mispairing between two nucleotide sequences; and the “completely reverse complementary” means that there is no mispairing between two nucleotide sequences.

16. The siRNA conjugate according to claim 15, wherein i) the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 3; the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 4; and the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is C; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is CC; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is CCC; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is ACCC; or

ii) the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 63; the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 64; and the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is U; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is GU; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is GGU; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is GGGU; or

iii) the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 123; the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 124; and the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is G; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AG; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UAG; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AUAG; or

iv) the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 183; the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 184; and the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is C; or

the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AC; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is GAC; or
 5 the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AGAC;

v) the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 243; the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 244; and the nucleotide sequence III and the nucleotide
 10 sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is G; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UG; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is CUG; or
 15 the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UCUG; or

vi) the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 303; the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 304; and the nucleotide sequence III and the nucleotide
 20 sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is G; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UG; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AUG; or
 25 the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UAUG.

17. The siRNA conjugate according to claim 16, wherein the nucleotide sequence III and the nucleotide sequence IV are completely reverse complementary to each other.
 30

18. The siRNA conjugate according to any one of claims 1-17, wherein the antisense strand further comprises a nucleotide sequence V, which has a length of 1 to 3 nucleotides and is linked to 3' terminal of the antisense strand, thereby forming a 3' overhang terminal of the antisense strand; or the nucleotide sequence V has a length of 2 nucleotides; or
 35 the nucleotide sequence V is 2 consecutive thymine deoxyribonucleotides or 2 consecutive uracil ribonucleotides; or the nucleotide sequence V is complementary to the nucleotides at the corresponding positions of the target mRNA.

19. The siRNA conjugate according to any one of claims 1-18, wherein the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 5, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 6; or
 40

the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 7, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 8; or
 45

the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 65, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 66; or

5 the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 67, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 68; or

the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 125, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 126; or

10 the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 127, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 128; or

the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 185, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 186; or

15 the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 187, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 188; or

20 the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 245, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 246; or

the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 247, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 248; or

25 the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 305, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 306; or

the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 307, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 308.

30 20. The siRNA conjugate according to any one of claims 11-19, wherein the siRNA has the nucleotide sequence as shown by siPCSKa1, siPCSKa2, siPCSKb1, siPCSKb2, siPCSKc1, siPCSKc2, siPCSKd1, siPCSKd2, siPCSKe1, siPCSKe2, siPCSKf1 or siPCSKf2.

35 21. The siRNA conjugate according to any one of claims 1-20, wherein at least one nucleotide in the sense strand or the antisense strand is a modified nucleotide, and/or at least one phosphate group is a phosphate group with modified group(s).

40 22. The siRNA conjugate according to claim 21, wherein each nucleotide in the sense strand and antisense strand is independently a fluoro modified nucleotide or a non-fluoro modified nucleotide; or

the fluoro modified nucleotides are located in the nucleotide sequence I and the nucleotide sequence II; and in the direction from 5' terminal to 3' terminal, the nucleotides at least at positions 7, 8 and 9 of the nucleotide sequence I are fluoro modified nucleotides; and in the direction from 5' terminal to 3' terminal, the nucleotides at least at positions 2, 6, 14, and 16 of the nucleotide sequence II are fluoro modified nucleotides; or

in the direction from 5' terminal to 3' terminal, the nucleotides at positions 7, 8 and 9 or at positions 5, 7, 8 and 9 of the nucleotide sequence I in the sense strand are fluoro modified nucleotides, and the nucleotides at the other positions in the sense strand are non-fluoro modified nucleotides; in the direction from 5' terminal to 3' terminal, the nucleotides at positions 2, 6, 14 and 16 or at positions 2, 6, 8, 9, 14, and 16 of the nucleotide sequence II in the antisense strand are fluoro modified nucleotides, and the nucleotides at the other positions in the antisense strand are non-fluoro modified nucleotides.

23. The siRNA conjugate according to claim 21 or 22, wherein each non-fluoro modified nucleotide is independently a nucleotide formed by substituting the 2'-hydroxy of the ribose group of a nucleotide with a non-fluoro group, or a nucleotide analogue; or

the nucleotide formed by substituting the 2'-hydroxy of the ribose group of a nucleotide with a non-fluoro group is selected from the group consisting of 2'-alkoxy modified nucleotide, 2'-substituted alkoxy modified nucleotide, 2'-alkyl modified nucleotide, 2'-substituted alkyl modified nucleotide, 2'-amino modified nucleotide, 2'-substituted amino modified nucleotide, and 2'-deoxy nucleotide; and the nucleotide analogue is selected from the group consisting of an isonucleotide, LNA, ENA, cET, UNA, and GNA; or

each non-fluoro modified nucleotide is a methoxy modified nucleotide, wherein the methoxy modified nucleotide refers to a nucleotide formed by substituting 2'-hydroxy of the ribose group with a methoxy group.

24. The siRNA conjugate according to any one of claims 21-23, wherein in the direction from 5' terminal to 3' terminal, the nucleotides at positions 5, 7, 8 and 9 of the nucleotide sequence I in the sense strand of the siRNA are fluoro modified nucleotides, and the nucleotides at the other positions in the sense strand of the siRNA are methoxy modified nucleotides; and in the direction from 5' terminal to 3' terminal, the nucleotides at positions 2, 6, 8, 9, 14 and 16 of the nucleotide sequence II in the antisense strand of the siRNA are fluoro modified nucleotides, and the nucleotides at the other positions in the antisense strand of the siRNA are methoxy modified nucleotides; or

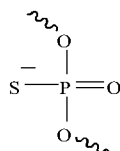
in the direction from 5' terminal to 3' terminal, the nucleotides at positions 5, 7, 8 and 9 of the nucleotide sequence I in the sense strand of the siRNA are fluoro modified nucleotides, and the nucleotides at the other positions of the sense strand of the siRNA are methoxy modified nucleotides; and in the direction from 5' terminal to 3' terminal, the nucleotides at positions 2, 6, 14 and 16 of the nucleotide sequence II in the antisense strand of the siRNA are fluoro modified nucleotides, and the nucleotides at the other positions in the antisense strand are methoxy modified nucleotides; or

in the direction from 5' terminal to 3' terminal, the nucleotides at positions 7, 8 and 9 of the nucleotide sequence I in the sense strand of the siRNA are fluoro modified nucleotides, and the nucleotides at the other positions in the sense strand of the siRNA are methoxy modified nucleotides; and in the direction from 5' terminal to 3' terminal, the nucleotides at positions 2, 6, 14 and 16 of the nucleotide sequence II in the antisense strand of the siRNA are fluoro modified nucleotides, and the nucleotides at the other positions in the antisense strand of the siRNA are methoxy modified nucleotides.

25. The siRNA conjugate according to any one of claims 1-24, wherein the siRNA is any one of siPCSKa1-M1, siPCSKa1-M2, siPCSKa1-M3, siPCSKa2-M1, siPCSKa2-M2, siPCSKa2-M3, siPCSKb1-M1, siPCSKb1-M2, siPCSKb1-M3, siPCSKb2-M1, siPCSKb2-M2, siPCSKb2-M3, siPCSKc1-M1, siPCSKc1-M2, siPCSKc1-M3, siPCSKc2-M1, siPCSKc2-M2, siPCSKc2-M3, siPCSKd1-M1,

siPCSKd1-M2, siPCSKd1-M3, siPCSKd2-M1, siPCSKd2-M2, siPCSKd2-M3, siPCSKe1-M1, siPCSKe1-M2, siPCSKe1-M3, siPCSKe2-M1, siPCSKe2-M2, siPCSKe2-M3, siPCSKf1-M1, siPCSKf1-M2, siPCSKf1-M3, siPCSKf2-M1, siPCSKf2-M2 or siPCSKf2-M3.

26. The siRNA conjugate according to claim 21, wherein the phosphate group with modified group(s) is a phosphorothioate group formed by substituting at least one oxygen atom in a phosphodiester bond in a phosphate group with a sulfur atom; or the phosphate group with modified group(s) is a phosphorothioate group having a structure as shown by Formula (1):



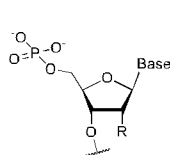
Formula (1).

27. The siRNA conjugate according to claim 26, wherein in the siRNA, the phosphorothioate linkage is located in at least one of the group consisting of the following positions:

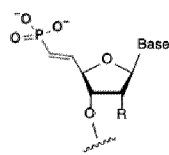
- the position between the first and second nucleotides at 5' terminal of the sense strand;
- the position between the second and third nucleotides at 5' terminal of the sense strand;
- the position between the first and second nucleotides at 3' terminal of the sense strand;
- the position between the second and third nucleotides at 3' terminal of the sense strand;
- the position between the first and second nucleotides at 5' terminal of the antisense strand;
- the position between the second and third nucleotides at 5' terminal of the antisense strand;
- the position between the first and second nucleotides at 3' terminal of the antisense strand; and
- the position between the second and third nucleotides at 3' terminal of the antisense strand.

28. The siRNA conjugate according to any one of claims 1-27, wherein the siRNA is any one of siPCSKa1-M1S, siPCSKa1-M2S, siPCSKa1-M3S, siPCSKa2-M1S, siPCSKa2-M2S, siPCSKa2-M3S, siPCSKb1-M1S, siPCSKb1-M2S, siPCSKb1-M3S, siPCSKb2-M1S, siPCSKb2-M2S, siPCSKb2-M3S, siPCSKc1-M1S, siPCSKc1-M2S, siPCSKc1-M3S, siPCSKc2-M1S, siPCSKc2-M2S, siPCSKc2-M3S, siPCSKd1-M1S, siPCSKd1-M2S, siPCSKd1-M3S, siPCSKd2-M1S, siPCSKd2-M2S, siPCSKd2-M3S, siPCSKe1-M1S, siPCSKe1-M2S, siPCSKe1-M3S, siPCSKe2-M1S, siPCSKe2-M2S, siPCSKe2-M3S, siPCSKf1-M1S, siPCSKf1-M2S, siPCSKf1-M3S, siPCSKf2-M1S, siPCSKf2-M2S or siPCSKf2-M3S.

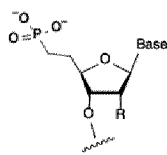
29. The siRNA conjugate according to any one of claims 1-28, wherein the nucleotide at 5'-terminal of the antisense strand of the siRNA is a 5'-phosphate nucleotide or a 5'-phosphate analogue modified nucleotide; or the 5'-phosphate nucleotide is a nucleotide having a structure as shown by Formula (2); and the 5'-phosphate analogue modified nucleotide is a nucleotide having a structure as shown by any one of Formulae (3) to (6):



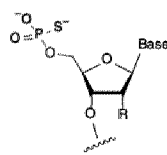
Formula (2)



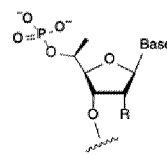
Formula (3)



Formula (4)



Formula (5)



Formula (6)

wherein R is selected from H, OH, methoxy or F;

“Base” represents a base selected from A, U, C, G, or T.

30. The siRNA conjugate according to any one of claims 1-29, wherein the siRNA is any one of siPCSKa1-M1P1, siPCSKa1-M2P1, siPCSKa1-M3P1, siPCSKa2-M1P1, siPCSKa2-M2P1, siPCSKa2-M3P1, siPCSKa1-M1SP1, siPCSKa1-M2SP1, siPCSKa1-M3SP1, siPCSKa2-M1SP1, siPCSKa2-M2SP1, siPCSKa2-M3SP1, siPCSKb1-M1P1, siPCSKb1-M2P1, siPCSKb1-M3P1, siPCSKb2-M1P1, siPCSKb2-M2P1, siPCSKb2-M3P1, siPCSKb1-M1SP1, siPCSKb1-M2SP1, siPCSKb1-M3SP1, siPCSKb2-M1SP1, siPCSKb2-M2SP1, siPCSKb2-M3SP1, siPCSKc1-M1P1, siPCSKc1-M2P1, siPCSKc1-M3P1, siPCSKc2-M1P1, siPCSKc2-M2P1, siPCSKc2-M3P1, siPCSKc1-M1SP1, siPCSKc1-M2SP1, siPCSKc1-M3SP1, siPCSKc2-M1SP1, siPCSKc2-M2SP1, siPCSKc2-M3SP1, siPCSKd1-M1P1, siPCSKd1-M2P1, siPCSKd1-M3P1, siPCSKd2-M1P1, siPCSKd2-M2P1, siPCSKd2-M3P1, siPCSKd1-M1SP1, siPCSKd1-M2SP1, siPCSKd1-M3SP1, siPCSKd2-M1SP1, siPCSKd2-M2SP1, siPCSKd2-M3SP1, siPCSKe1-M1P1, siPCSKe1-M2P1, siPCSKe1-M3P1, siPCSKe2-M1P1, siPCSKe2-M2P1, siPCSKe2-M3P1, siPCSKe1-M1SP1, siPCSKe1-M2SP1, siPCSKe1-M3SP1, siPCSKe2-M1SP1, siPCSKe2-M2SP1, siPCSKe2-M3SP1, siPCSKf1-M1P1, siPCSKf1-M2P1, siPCSKf1-M3P1, siPCSKf2-M1P1, siPCSKf2-M2P1, siPCSKf2-M3P1, siPCSKf1-M1SP1, siPCSKf1-M2SP1, siPCSKf1-M3SP1, siPCSKf2-M1SP1, siPCSKf2-M2SP1 or siPCSKf2-M3SP1.

31. An siRNA comprising a sense strand and an antisense strand; each nucleotide in the sense strand and antisense strand is independently a fluoro modified or non-fluoro modified nucleotide; wherein the sense strand comprises a nucleotide sequence I, and the antisense strand comprises a nucleotide sequence II; the nucleotide sequence I and the nucleotide sequence II are at least partly reverse complementary to form a double-stranded region; the fluoro modified nucleotides are located in the nucleotide sequence I and the nucleotide sequence II; and in the direction from 5' terminal to 3' terminal, the nucleotides at positions 7, 8 and 9 of the nucleotide sequence I in the sense strand are fluoro modified nucleotides, and the nucleotides at the other positions in the sense strand are non-fluoro modified nucleotides; in the direction from 5' terminal to 3' terminal, the nucleotides at positions 2, 6, 14, and 16 of the nucleotide sequence II in the antisense strand are fluoro modified nucleotides, and the nucleotides at the other positions in the antisense strand are non-fluoro modified nucleotides; and

i) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 1 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 2 have an equal length and no more than 3 nucleotide differences; the nucleotide sequence I comprises a nucleotide Z_3 at the position corresponding to Z_1 ; the nucleotide sequence II comprises a nucleotide Z_4 at the position corresponding to Z_2 , wherein Z_4 is the first nucleotide at 5' terminal of the antisense strand; or

ii) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 61 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 62 have an equal length and no more than 3 nucleotide differences; the nucleotide sequence I comprises a nucleotide Z_7 at the position corresponding to Z_5 ; the nucleotide sequence II comprises a nucleotide Z_8 at the position corresponding to Z_6 , wherein Z_8 is the first nucleotide at 5' terminal of the antisense strand; or

iii) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 121 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 122 have an equal length and no more than 3 nucleotide differences; the nucleotide sequence

I comprises a nucleotide Z_{11} at the position corresponding to Z_9 ; the nucleotide sequence II comprises a nucleotide Z_{12} at the position corresponding to Z_{10} , wherein Z_{12} is the first nucleotide at 5' terminal of the antisense strand; or

5 iv) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 181 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 182 have an equal length and no more than 3 nucleotide differences; the nucleotide sequence I comprises a nucleotide Z_{15} at the position corresponding to Z_{13} ; the nucleotide sequence II comprises a nucleotide Z_{16} at the position corresponding to Z_{14} , wherein Z_{16} is the first nucleotide at 5' terminal of the
10 antisense strand; or

v) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 241 have an equal length and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 242 have an equal length and no more than 3 nucleotide differences; the nucleotide sequence
15 I comprises a nucleotide Z_{19} at the position corresponding to Z_{17} ; the nucleotide sequence II comprises a nucleotide Z_{20} at the position corresponding to Z_{18} , wherein Z_{20} is the first nucleotide at 5' terminal of the antisense strand; or

vi) the nucleotide sequence I and the nucleotide sequence as shown by SEQ ID NO: 301 have an equal length
20 and no more than 3 nucleotide differences, and the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 302 have an equal length and no more than 3 nucleotide differences; the nucleotide sequence I comprises a nucleotide Z_{23} at the position corresponding to Z_{21} ; the nucleotide sequence II comprises a nucleotide Z_{24} at the position corresponding to Z_{22} , wherein Z_{24} is the first nucleotide at 5' terminal of the antisense strand.
25

32. The siRNA according to claim 31, wherein each non-fluoro modified nucleotide is independently a nucleotide formed by substituting the 2'-hydroxy of the ribose group of a nucleotide with a non-fluoro group, or a nucleotide analogue.

30 33. The siRNA according to claim 32, wherein the nucleotide formed by substituting the 2'-hydroxy of the ribose group of a nucleotide with a non-fluoro group is selected from the group consisting of 2'-alkoxy modified nucleotide, 2'-substituted alkoxy modified nucleotide, 2'-alkyl modified nucleotide, 2'-substituted alkyl modified nucleotide, 2'-amino modified nucleotide, 2'-substituted amino modified nucleotide, and 2'-deoxy nucleotide; and the nucleotide analogue is selected from the group consisting of an isonucleotide, LNA,
35 ENA, cET, UNA, and GNA.

34. The siRNA according to any one of claims 31-33, wherein each non-fluoro modified nucleotide is a methoxy modified nucleotide, wherein the methoxy modified nucleotide refers to a nucleotide formed by substituting 2'-hydroxy of the ribose group of a nucleotide with a methoxy group.
40

35. The siRNA according to any one of claims 31-34, wherein i) the nucleotide sequence I has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 1, and/or the nucleotide sequence II has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 2; or
45

ii) the nucleotide sequence I has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 61, and/or the nucleotide sequence II has no more than 1 nucleotide difference from the

nucleotide sequence as shown by SEQ ID NO: 62; or

iii) the nucleotide sequence I has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 121, and/or the nucleotide sequence II has no more than 1 nucleotide difference from the
5 nucleotide sequence as shown by SEQ ID NO: 122; or

iv) the nucleotide sequence I has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 181, and/or the nucleotide sequence II has no more than 1 nucleotide difference from the
10 nucleotide sequence as shown by SEQ ID NO: 182; or

v) the nucleotide sequence I has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 241, and/or the nucleotide sequence II has no more than 1 nucleotide difference from the
nucleotide sequence as shown by SEQ ID NO: 242; or

vi) the nucleotide sequence I has no more than 1 nucleotide difference from the nucleotide sequence as shown by SEQ ID NO: 301, and/or the nucleotide sequence II has no more than 1 nucleotide difference from the
15 nucleotide sequence as shown by SEQ ID NO: 302.

36. The siRNA according to any one of claims 31-35, wherein i) the nucleotide difference between the
20 nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 2 includes a difference at the position Z_4 , and Z_4 is selected from A, U or C; or

ii) the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 62 includes a difference at the position Z_8 , wherein Z_8 is selected from A, C or G; or
25

iii) the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 122 includes a difference at the position Z_{12} , wherein Z_{12} is selected from A, C or G; or

iv) the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 182 includes a difference at the position Z_{16} , wherein Z_{16} is selected from U, C or G; or
30

v) the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 242 includes a difference at the position Z_{20} , wherein Z_{20} is selected from U, C or G; or

vi) the nucleotide difference between the nucleotide sequence II and the nucleotide sequence as shown by SEQ ID NO: 302 includes a difference at the position Z_{24} , wherein Z_{24} is selected from A, C or G.
35

37. The siRNA according to claim 36, wherein Z_3 is a nucleotide complementary to Z_4 ; or
 Z_7 is a nucleotide complementary to Z_8 ; or
40 Z_{11} is a nucleotide complementary to Z_{12} ; or
 Z_{15} is a nucleotide complementary to Z_{16} ; or
 Z_{19} is a nucleotide complementary to Z_{20} ; or
 Z_{23} is a nucleotide complementary to Z_{24} .

38. The siRNA according to any one of claims 31-37, wherein the nucleotide sequence I and the nucleotide sequence II are basically reverse complementary, substantially reverse complementary, or completely reverse
45

complementary to each other; the “basically reverse complementary” means that there is no more than 3 base mispairings between two nucleotide sequences; the “substantially reverse complementary” means that there is no more than 1 base mispairing between two nucleotide sequences; and the “completely reverse complementary” means that there is no mispairing between two nucleotide sequences.

5

39. The siRNA according to any one of claims 31-38, wherein the sense strand further comprises a nucleotide sequence III, the antisense strand further comprises a nucleotide sequence IV; and the nucleotide sequence III and the nucleotide sequence IV independently of one another have a length of 1 to 4 nucleotides; the nucleotide sequence III is linked to 5' terminal of the nucleotide sequence I, the nucleotide sequence IV is linked to 3' terminal of the nucleotide sequence II; the nucleotide sequence III and the nucleotide sequence IV have an equal length, and are substantially reverse complementary or completely reverse complementary to each other; the “substantially reverse complementary” means that there is no more than 1 base mispairing between two nucleotide sequences; and the “completely reverse complementary” means that there is no mispairing between two nucleotide sequences.

15

40. The siRNA according to any one of claims 31-39, wherein i) the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 3; the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 4; and the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is C; or
the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is CC; or
the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is CCC; or
the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is ACCC; or

25

ii) the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 63; the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 64; and the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is U; or
the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is GU; or
the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is GGU; or
the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is GGGU; or

35

iii) the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 123; the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 124; and the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is G; or
the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AG; or
the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UAG; or
the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AUAG; or

45

iv) the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 183; the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 184; and the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is C; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AC; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is GAC; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AGAC; or

10

v) the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 243; the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 244; and the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is G; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UG; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is CUG; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UCUG; or

20

vi) the nucleotide sequence I is the nucleotide sequence as shown by SEQ ID NO: 303; the nucleotide sequence II is the nucleotide sequence as shown by SEQ ID NO: 304; and the nucleotide sequence III and the nucleotide sequence IV both have a length of 1 nucleotide, and the base of the nucleotide sequence III is G; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 2 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UG; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 3 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is AUG; or the nucleotide sequence III and the nucleotide sequence IV both have a length of 4 nucleotides, and in the direction from 5' terminal to 3' terminal, the base composition of the nucleotide sequence III is UAUG.

30

41. The siRNA according to claim 40, wherein the nucleotide sequence III and the nucleotide sequence IV are completely reverse complementary to each other.

42. The siRNA according to any one of claims 31-41, wherein the antisense strand further comprises nucleotide sequence V, which has a length of 1 to 3 nucleotides and is linked to 3' terminal of the antisense strand, thereby forming a 3' overhang terminal of the antisense strand; or the nucleotide sequence V has a length of 2 nucleotides; or the nucleotide sequence V is 2 consecutive thymine deoxyribonucleotides or 2 consecutive uracil ribonucleotides; or the nucleotide sequence V is complementary to the nucleotides at the corresponding positions of the target mRNA.

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43. The siRNA according to any one of claims 31-42, wherein the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 5, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 6; or

40

the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 7, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 8; or

5 the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 65, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 66; or

the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 67, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 68; or

10 the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 125, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 126; or

the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 127, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 128; or

15 the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 185, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 186; or

20 the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 187, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 188; or

the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 245, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 246; or

25 the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 247, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 248; or

the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 305, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 306; or

30 the sense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 307, and the antisense strand of the siRNA comprises the nucleotide sequence as shown by SEQ ID NO: 308.

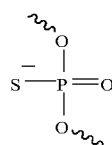
35 44. The siRNA according to any one of claims 31-43, wherein the siRNA has the nucleotide sequence as shown by siPCSKa1, siPCSKa2, siPCSKb1, siPCSKb2, siPCSKc1, siPCSKc2, siPCSKd1, siPCSKd2, siPCSKe1, siPCSKe2, siPCSKf1 or siPCSKf2.

40 45. The siRNA according to any one of claims 31-44, wherein the siRNA is any one of siPCSKa1-M1, siPCSKa2-M1, siPCSKb1-M1, siPCSKb2-M1, siPCSKc1-M1, siPCSKc2-M1, siPCSKd1-M1, siPCSKd2-M1, siPCSKe1-M1, siPCSKe2-M1, siPCSKf1-M1 or siPCSKf2-M1.

46. The siRNA according to any one of claims 31-45, wherein at least one phosphate group in the sense strand or the antisense strand is a phosphate group with modified group(s).

45 47. The siRNA according to claim 46, wherein the phosphate group with modified group(s) is a phosphorothioate group formed by substituting at least one oxygen atom in a phosphodiester bond in the

phosphate group with a sulfur atom; or the phosphate group with modified group(s) is a phosphorothioate group having a structure as shown by Formula (1):



Formula (1).

5

48. The siRNA according to claim 47, wherein in the siRNA, the phosphorothioate linkage is located in at least one of the group consisting of the following positions:

the position between the first and second nucleotides at 5' terminal of the sense strand;

the position between the second and third nucleotides at 5' terminal of the sense strand;

10 the position between the first and second nucleotides at 3' terminal of the sense strand;

the position between the second and third nucleotides at 3' terminal of the sense strand;

the position between the first and second nucleotides at 5' terminal of the antisense strand;

the position between the second and third nucleotides at 5' terminal of the antisense strand;

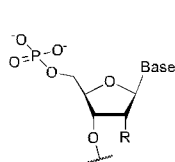
the position between the first and second nucleotides at 3' terminal of the antisense strand; and

15 the position between the second and third nucleotides at 3' terminal of the antisense strand.

49. The siRNA according to any one of claims 31-48, wherein the siRNA is any one of siPCSKa1-M1S, siPCSKa2-M1S, siPCSKb1-M1S, siPCSKb2-M1S, siPCSKc1-M1S, siPCSKc2-M1S, siPCSKd1-M1S, siPCSKd2-M1S, siPCSKe1-M1S, siPCSKe2-M1S, siPCSKf1-M1S or siPCSKf2-M1S.

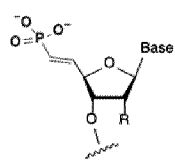
20

50. The siRNA according to any one of claims 31-49, wherein the nucleotide at 5'-terminal of the antisense strand of the siRNA is a 5'-phosphate nucleotide or a 5'-phosphate analogue modified nucleotide; or the 5'-phosphate nucleotide is a nucleotide having a structure as shown by Formula (2); and the 5'-phosphate analogue modified nucleotide is a nucleotide having a structure as shown by any one of Formulae (3) to (6):

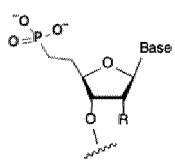


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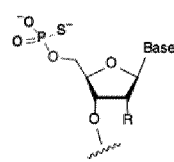
Formula (2)



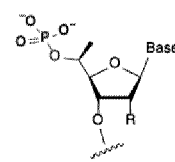
Formula (3)



Formula (4)



Formula (5)



Formula (6)

wherein R is selected from H, OH, methoxy, or F;

30 "Base" represents a base selected from A, U, C, G, or T.

51. The siRNA according to any one of claims 31-50, wherein the siRNA is any one of siPCSKa1-M1P1, siPCSKa2-M1P1, siPCSKa1-M1SP1, siPCSKa2-M1SP1, siPCSKb1-M1P1, siPCSKb2-M1P1, siPCSKb1-M1SP1, siPCSKb2-M1SP1, siPCSKc1-M1P1, siPCSKc2-M1P1, siPCSKc1-M1SP1, siPCSKc2-M1SP1, siPCSKd1-M1P1, siPCSKd2-M1P1, siPCSKd1-M1SP1, siPCSKd2-M1SP1, siPCSKe1-M1P1, siPCSKe2-M1P1, siPCSKe1-M1SP1, siPCSKe2-M1SP1, siPCSKf1-M1P1, siPCSKf2-M1P1, siPCSKf1-M1SP1 or siPCSKf2-M1SP1.

35

52. The siRNA according to any one of claims 31-51, wherein the siRNA is any one of siPCSKa1-M1SP, siPCSKb1-M1SP, siPCSKc1-M1SP, siPCSKd1-M1SP, siPCSKe1-M1SP or siPCSKf1-M1SP.

40

53. A pharmaceutical composition, comprising the siRNA according to any one of claims 31-52, and a pharmaceutically acceptable carrier.
- 5 54. An siRNA conjugate, comprising the siRNA according to any one of claims 31-52, and a conjugation group conjugatively linked to the siRNA.
55. Use of the siRNA conjugate according to any one of claims 1-30 and 54, the siRNA according to any one of claims 31-52 and/or the pharmaceutical composition according to claim 53 in the manufacture of a
 10 medicament for treating and/or preventing diseases or physiological conditions caused by abnormal expression of PCSK9 gene.
56. A method for treating and/or preventing diseases or physiological conditions caused by abnormal expression of PCSK9 gene, comprising administering an effective amount of the siRNA conjugate according to any one
 15 of claims 1-30 and 54, the siRNA according to any one of claims 31-52 and/or the pharmaceutical composition according to claim 53 to a subject suffering from the diseases or physiological conditions.
57. A method for inhibiting the expression of PCSK9 gene in hepatocytes, comprising contacting an effective amount of the siRNA conjugate according to any one of claims 1-30 and 54, the siRNA according to any one
 20 of claims 31-52 and/or the pharmaceutical composition according to claim 53 with the hepatocytes.
58. A kit, comprising the siRNA conjugate according to any one of claims 1-30 and 54, the siRNA according to any one of claims 31-52 and/or the pharmaceutical composition according to claim 53.

25

FIGURES

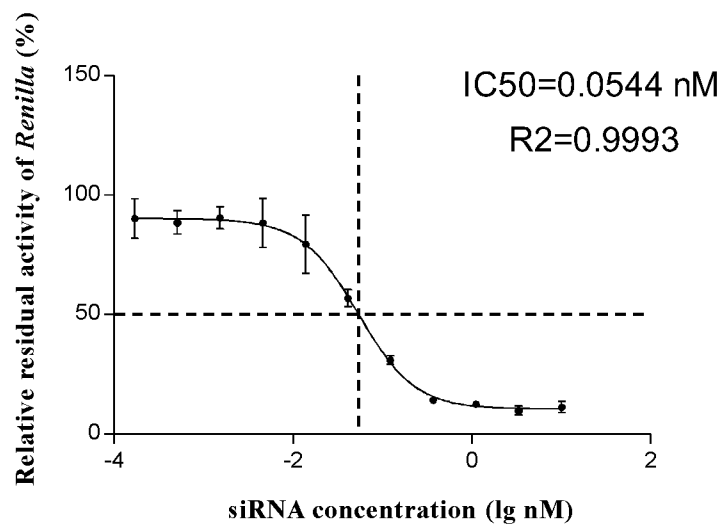


Figure 1A

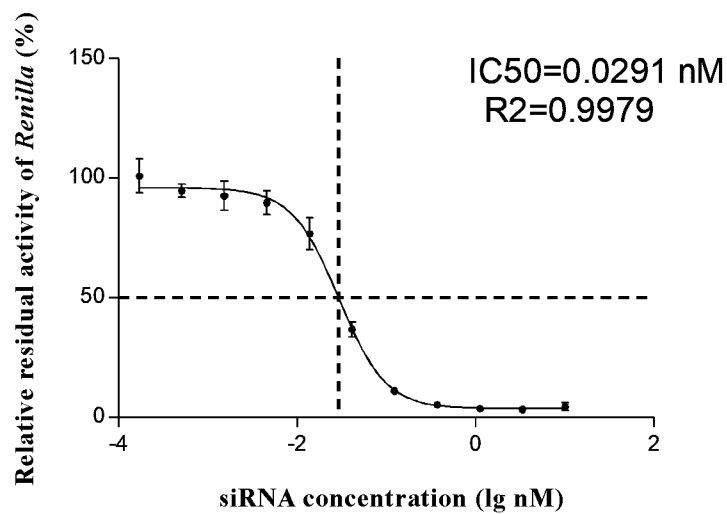


Figure 1B

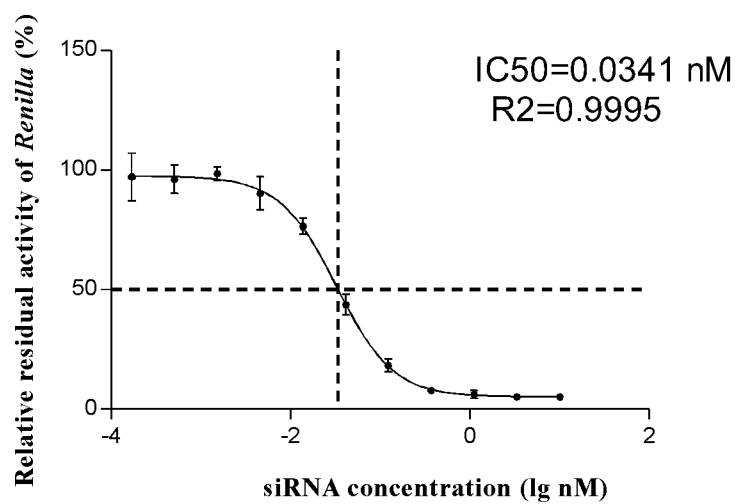


Figure 1C

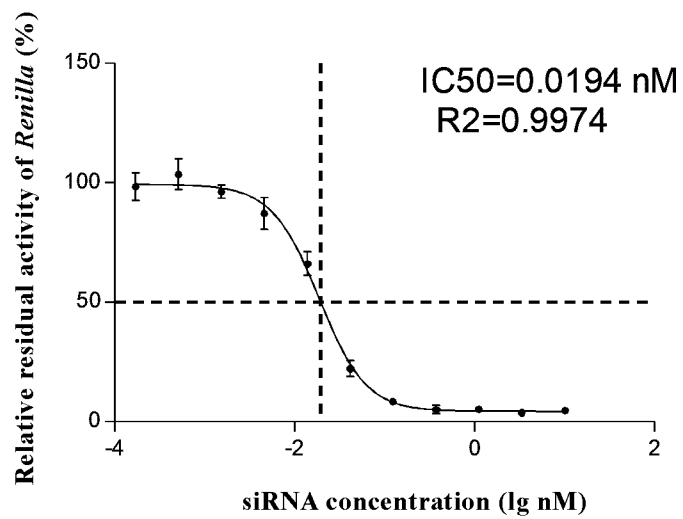


Figure 1D

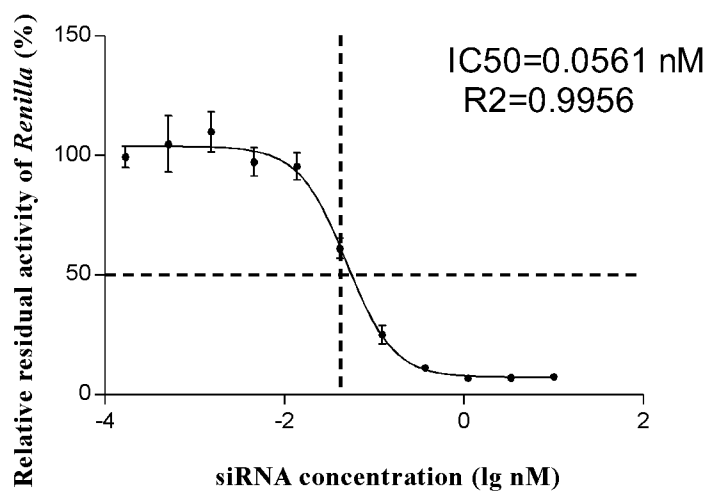


Figure 1E

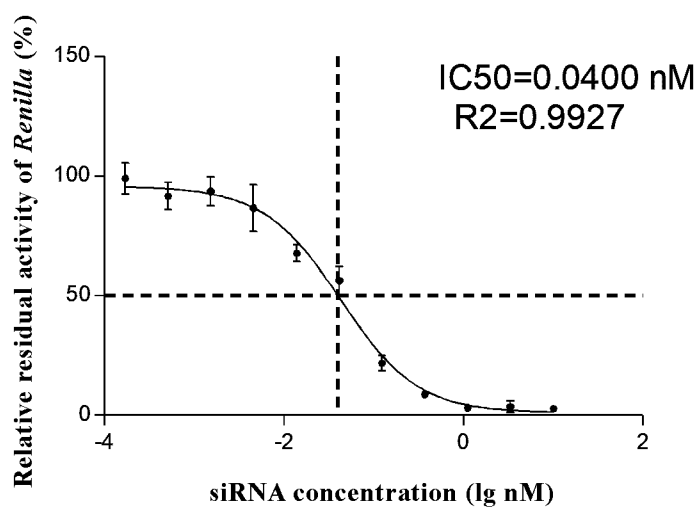


Figure 1F

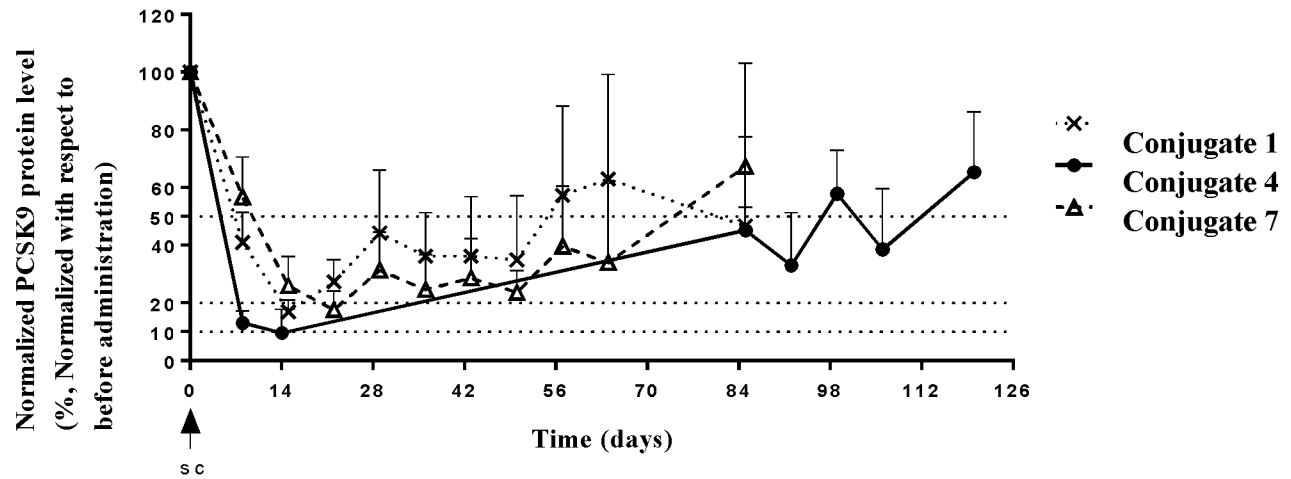


Figure 2

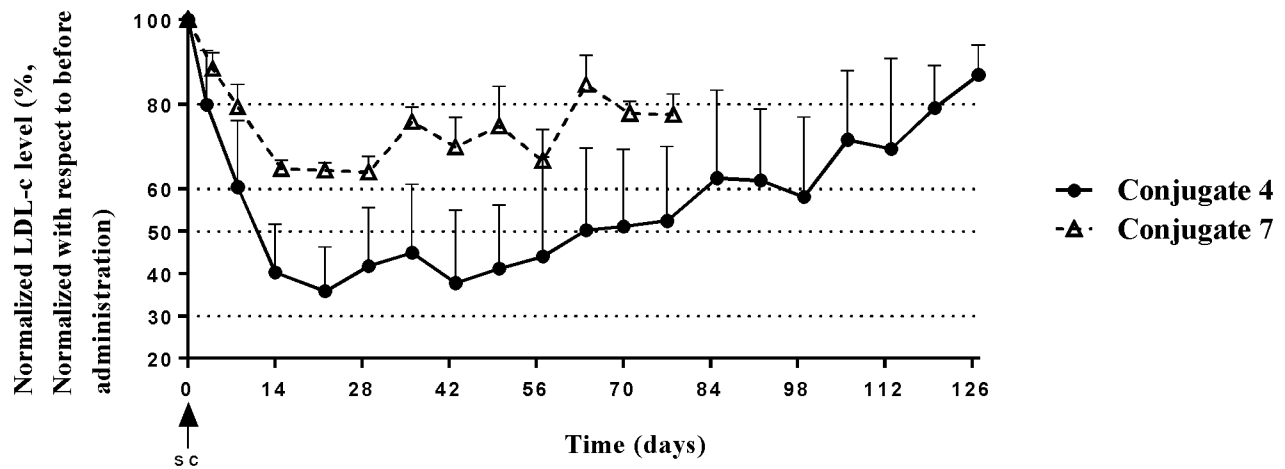


Figure 3

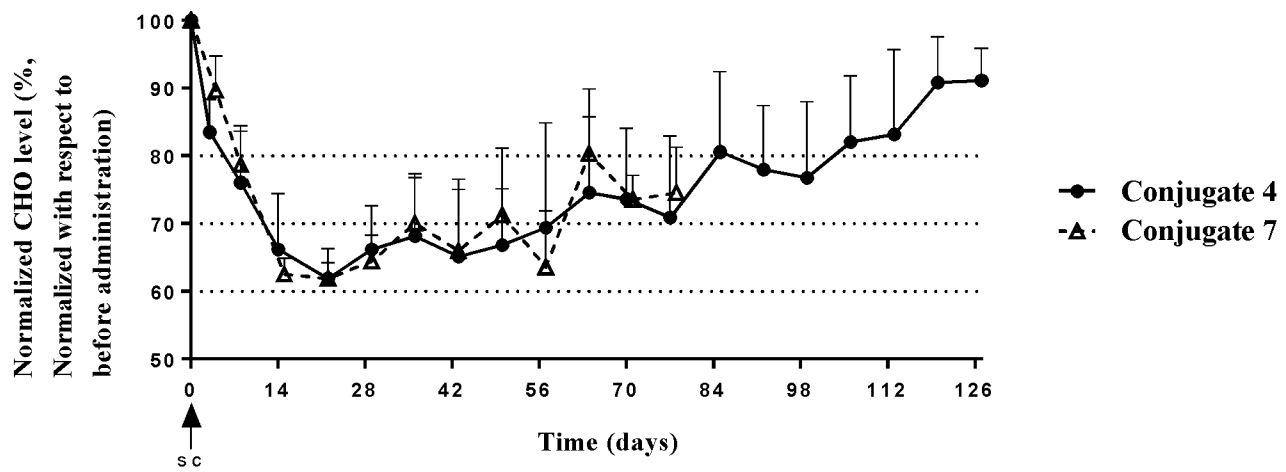


Figure 4

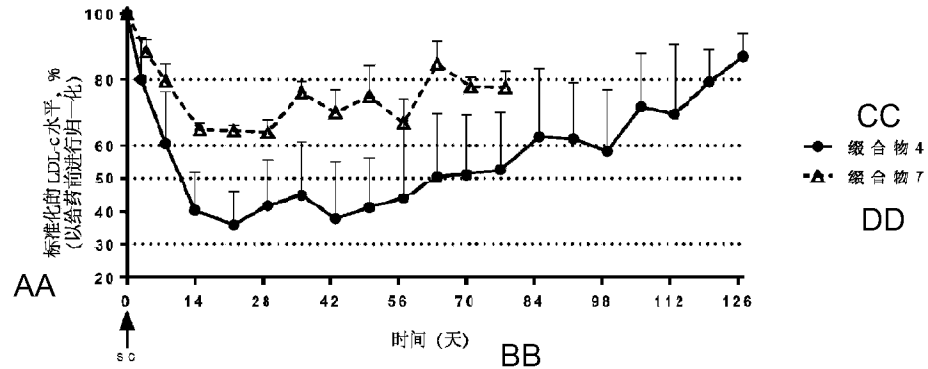


图 3

AA Standardized LDL-c level, % (normalized with pre-administration)

BB Time (days)

CC Conjugate 4

DD Conjugate 7