

DOUBLE-STRANDED RNAi AGENTS FOR TARGETING AND REGULATING HBV GENE EXPRESSION, AND A USE THEREOF

SPECIFICATION

TECHNICAL FIELD

[0001] The present disclosure relates to the field of nucleic acid modification technology, and specifically, to a double-stranded RNAi agent for targeting and regulating HBV gene expression, and a use thereof.

BACKGROUND

[0002] Nucleic acid drugs, particularly oligonucleotide drugs, have been widely used due to their simple synthesis and high activity. Oligonucleotide drugs typically include antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), microRNAs (miRNAs), and nucleic acid aptamers, among others.

[0003] Oligonucleotides are short DNA or RNA molecules or oligomers that can easily bind to their complementary oligonucleotides, DNA, or RNA in a sequence-specific manner to form duplexes or, less commonly, hybrids. This fundamental property enables oligonucleotides to have broad applications in gene detection, research, and medicine. In nature, oligonucleotides are often small RNA molecules that play a role in the regulation of gene expression or are intermediates derived from the degradation of larger nucleic acid molecules.

[0004] RNA interference (RNAi) is a natural defense mechanism against exogenous genes. siRNA can downregulate target genes by recognizing specific sequences and degrading the target mRNA.

[0005] An effective molecule of a classical RNAi consists of a characteristic 19 + 2 nucleotide polymer structure (a double-helix structure composed of a 21-nucleotide RNA molecule paired with a 19-nucleotide molecule of complementary bases, comprising a 3' end overhang of two nucleotides). One strand (the guide strand or antisense strand) of siRNA is complementary to the target gene's transcript mRNA, while the other strand is designated as the passenger strand (or sense strand). The siRNA (antisense strand) guides the Argonaute protein (AGO2) to complementary to the target transcript and becomes part of the RNA-induced silencing

complex (RISC). The complete complementarity between the siRNA (antisense strand) and the target leads to the cleavage of the target transcript at positions 10–11 corresponding to the guide strand (antisense strand), catalyzed by the AGO2 protein.

[0006] siRNA possesses inherent advantages compared to small molecules and antibody drugs, as siRNA executes its function through Watson–Crick base pairing with mRNA, whereas small molecules and monoclonal antibody drugs require recognition of the complex three-dimensional structure of specific proteins. Therefore, many diseases cannot be treated with small molecules or monoclonal antibodies because their target molecules are highly active and lack identifiable molecular structures with sufficient affinity and binding specificity. The mechanism of action of siRNA drugs enables them to regulate the expression of target proteins at the genetic level, offering superior target specificity compared to small molecules or antibody drugs. The principle of base complementarity also allows siRNA to have a broader therapeutic range, simpler design, and shorter development cycles.

[0007] In natural oligonucleotides, nucleotides are linked by phosphodiester bonds, making them highly susceptible to nucleases under physiological conditions. As a result, natural, unmodified oligonucleotide drugs are rapidly degraded by nucleases in vivo, exhibit low activity, and have poor druggability. Chemical modification of oligonucleotide structures is an effective approach to enhance their activity. Such modifications can improve their stability against nucleases, increase their affinity for RNA, and promote cellular uptake and tissue targeting, thereby effectively regulating the expression of target genes.

[0008] Based on the basic structure of oligonucleotides—comprising the nucleobase, sugar ring, phosphate backbone, and terminal—chemical modifications can be performed in four parts:

[0009] 1) Nucleobase modifications, which primarily include purine modifications, pyrimidine modifications, and nucleobase replacements. Purine modifications include N6-methyladenosine, N1-methyladenosine, and 7-methylguanosine modifications. Pyrimidine modifications include 3-methyluridine, 5-methyluridine, 5-methylcytidine, N4-acetylcytidine, pseudouridine, thiouridine, propynyluridine, and dihydrouridine modifications, among others.

[0010] 2) Sugar ring modifications, which mainly include modifications and replacements of the sugar moiety. Sugar ring modifications include 2'-, 4'-, and 5'-modifications, isomer

modifications, and combinations thereof. Among siRNA modifications, the most common 2'-modifications are 2'-OMe (2'-methoxy) and 2'-F (2'-fluoro). Compared to natural siRNA, siRNA modified with both 2'-OMe and 2'-F exhibits higher T_m values, stronger serum stability, and improved activity.

[0011] 3) Phosphate backbone modifications, which primarily include phosphorothioate modifications; methylphosphonate, selenophosphate, boranophosphate, and dithiophosphate modifications, as well as the replacement of bridging oxygen atoms in the phosphodiester linkage with sulfur atoms; the phosphate group between nucleotides be entirely replaced with non-phosphorus groups, such as replacing the phosphorus atom with carbon (C), sulfur (S), or nitrogen (N) atoms, forming guanidinium or S-methylthiourea groups, among others.

[0012] 4) Terminal modifications, which involve the covalent conjugation of special groups to the 5' end or/and 3' end of the sense strand, as well as phosphorylation modifications at the 5' end of the antisense strand.

[0013] Hepatitis B virus (HBV) is a double-stranded hepatotropic virus that infects only humans and non-human primates. It primarily replicates in the liver and can be transmitted through maternal-neonatal transmission, blood (including minor skin and mucosal injuries), and sexual contact. HBV infection remains a major global health issue. Chronic HBV infection has a high probability of progressing to liver fibrosis, cirrhosis, and liver cancer.

[0014] The current standard of care for chronic HBV infection involves treatment with oral nucleos(t)ide analogs (NAs) and injections of interferon-alpha. NAs inhibit HBV replication by suppressing HBV DNA synthesis. However, most patients require long-term treatment, and the rate of virological relapse after discontinuation of therapy is high. Interferon-alpha exerts dual effects of immunomodulation and antiviral activity by enhancing immune cell function, promoting cytokine expression, and inducing interferon signaling to encode multiple antiviral proteins. Interferon-alpha is effective only in a subset of patients when used alone and is associated with relatively poor tolerability. In the "Guidelines for the Prevention and Treatment of Chronic Hepatitis B (version 2022)", Entecavir, Tenofovir Disoproxil Fumarate, Tenofovir Alafenamide Fumarate, and Tenofovir Amibufenamide are recommended for nucleos (t) ide analogs, and pegylated interferon-alpha is recommended for α interferons.

[0015] The current treatment goal for chronic HBV infection is to achieve a functional cure,

defined as sustained undetectable levels of HBV DNA and HBV surface antigen (HBsAg) after discontinuation of therapy, with or without HBsAg seroconversion. A sustained reduction in HBsAg levels and seroconversion are critical for achieving a functional cure. This is expected to alleviate liver inflammation, improve liver histopathology, reduce the incidence of end-stage liver disease, and prolong patient survival. Currently, achieving a functional cure through clinical drug therapy is difficult, or with a very low functional cure rate. Therefore, there is a need for further research and development of drugs that can downregulate HBsAg expression to achieve a functional cure.

CONTENT OF THE PRESENT INVENTION

[0016] To address the technical problem in the prior art of lacking a drug capable of more effectively inhibiting HBV gene expression, the present disclosure provides a double-stranded RNAi agent for targeting and regulating HBV gene expression, and a use thereof. By targeting the HBV genome sequence, a series of unique siRNA sequences are designed and modified with specific templates in the present disclosure.

[0017] Typically, siRNA is modified with 2'-methoxy (2'-OMe) and 2'-fluoro (2'-F) on the monomers. However, even when considering only the combination of these two monomer modifications, for an siRNA with 44 nucleotides in total (including both the sense and antisense strands), there are 2^{44} possible modification combinations. Furthermore, the addition of different terminal phosphorothioate modification patterns significantly increases the number of possible modification patterns.

[0018] The activity of the same siRNA sequence can vary greatly depending on the modification pattern, and different siRNA sequences with the same modification pattern can also exhibit significant differences in activity. Although there are some general principles for siRNA modification design, existing research has shown that it is not possible to accurately predict activity based on modification patterns, as there is no deterministic relationship between modification patterns and activity. Therefore, screening for highly active modification patterns from an enormous number of possible combinations is extremely challenging.

[0019] Through chemical modifications of the designed siRNA sequences, specific modified sequences that exhibit significant inhibitory effects on HBV gene expression were identified in

the present disclosure.

[0020] In one aspect, the present disclosure provides a double-stranded RNAi agent, wherein the double-stranded RNAi agent comprises an antisense strand and a sense strand complementary to the antisense strand forming a duplex region, wherein the nucleotide sequence of the antisense strand is as shown in SEQ ID NO: 8, 11, or 13, or the nucleotide sequence of the antisense strand is a modified sequence of the sequence shown in SEQ ID NO: 8, 11, or 13.

[0021] In another aspect, the present disclosure further provides a conjugate comprising the double-stranded RNAi agent and a ligand conjugated to the double-stranded RNAi agent.

[0022] In another aspect, the present disclosure further provides a pharmaceutical composition comprising the double-stranded RNAi agent or the conjugate, along with a pharmaceutically acceptable carrier.

[0023] In another aspect, the present disclosure further provides a kit, wherein the kit comprises a kit A, and the kit A comprises one or more of the double-stranded RNAi agent, the conjugate, or the pharmaceutical composition.

[0024] In another aspect, the present disclosure further provides a use of the double-stranded RNAi agent, the conjugate, or the pharmaceutical composition in the preparation of a medicament for preventing and/or treating a disease associated with HBV gene expression.

[0025] In another aspect, the present disclosure further provides a method for reducing HBV gene expression or inhibiting HBV replication for non-preventive and/or non-therapeutic purposes, wherein the method comprises administering to a sample one or more of the double-stranded RNAi agent, the conjugate, the pharmaceutical composition, or the kit.

[0026] Based on common knowledge in the field, the above preferred conditions can be combined in any manner to obtain various preferred embodiments of the present disclosure.

[0027] All reagents and raw materials used in the present disclosure are commercially available.

[0028] The positive and progressive effects of the present disclosure are as follows:

[0029] (1) The sequences or modified sequences of the present application exhibit significant inhibitory effects on HBsAg, demonstrating superior performance compared to the positive control sequences.

[0030] (2) When the modified sequences are conjugated with GalNAc compounds, they can be efficiently delivered to the liver of animals, significantly inhibiting HBV gene expression and markedly reducing serum levels of HBsAg, HBeAg, and HBV DNA.

[0031] (3) The sequences modified using the modification templates of the present disclosure show significantly improved HBV inhibitory activity compared to sequences disclosed in the prior art that are relatively similar.

[0032] (4) The present disclosure finds that siRNAs with similar sequences can exhibit vastly different levels of activity.

[0033] (5) The present disclosure also finds that different sequences exhibit varying degrees of sensitivity to different modification templates. It is uncertain which modification template, when applied to an siRNA sequence, will result in high activity.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] FIG. 1A shows serum HBsAg levels in AAV-HBV model mice at a dose of 3 mg/kg of the candidate siRNA sequence.

[0035] FIG. 1B shows serum HBV DNA levels in AAV-HBV model mice at a dose of 3 mg/kg of the candidate siRNA sequence.

[0036] FIG. 1C shows serum HBeAg levels in AAV-HBV model mice at a dose of 3 mg/kg of the candidate siRNA sequence.

[0037] FIG. 1D shows serum HBsAb levels in AAV-HBV model mice at a dose of 3 mg/kg of the candidate siRNA sequence.

[0038] FIG. 1E shows serum ALT levels in AAV-HBV model mice at a dose of 3 mg/kg of the candidate siRNA sequence.

[0039] FIG. 1F shows body weight changes in AAV-HBV model mice at a dose of 3 mg/kg of the candidate siRNA sequence.

[0040] FIG. 2A shows serum HBsAg levels in mice after secondary viral challenge.

[0041] FIG. 2B shows serum HBV DNA levels in mice after secondary viral challenge.

[0042] FIG. 2C shows serum HBeAg levels in mice after secondary viral challenge.

[0043] FIG. 2D shows serum HBbAg levels in mice after secondary viral challenge.

[0044] FIG. 2E shows serum ALT levels in mice after secondary viral challenge.

- [0045]** FIG. 2F shows body weight changes in mice after secondary viral challenge.
- [0046]** FIG. 3A shows HBsAg levels in AAV-HBV model mice at a dose of 0.5 mg/kg of the candidate siRNA sequence.
- [0047]** FIG. 3B shows HBV DNA levels in AAV-HBV model mice at a dose of 0.5 mg/kg of the candidate siRNA sequence.
- [0048]** FIG. 3C shows HBeAg levels in AAV-HBV model mice at a dose of 0.5 mg/kg of the candidate siRNA sequence.
- [0049]** FIG. 3D shows HBsAb levels in AAV-HBV model mice at a dose of 0.5 mg/kg of the candidate siRNA sequence.
- [0050]** FIG. 3E shows ALT levels in AAV-HBV model mice at a dose of 0.5 mg/kg of the candidate siRNA sequence.
- [0051]** FIG. 3F shows body weight changes in AAV-HBV model mice at a dose of 0.5 mg/kg of the candidate siRNA sequence.
- [0052]** FIG. 4A shows HBsAg levels in AAV-HBV model mice treated with candidate siRNA sequences conjugated to different GalNAc moieties.
- [0053]** FIG. 4B shows HBV DNA levels in AAV-HBV model mice treated with candidate siRNA sequences conjugated to different GalNAc moieties.
- [0054]** FIG. 4C shows HBeAg levels in AAV-HBV model mice treated with candidate siRNA sequences conjugated to different GalNAc moieties.
- [0055]** FIG. 4D shows ALT levels in AAV-HBV model mice treated with candidate siRNA sequences conjugated to different GalNAc moieties.
- [0056]** FIG. 4E shows body weight changes in AAV-HBV model mice treated with candidate siRNA sequences conjugated to different GalNAc moieties.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

- [0057]** To make the present disclosure more easily understood, certain terms are first defined. Additionally, it should be noted that whenever a value or a range of values for a parameter is listed, the purpose is to indicate that intermediate values and ranges of these referenced values are also part of the present disclosure.
- [0058]** As used herein, the articles “a” and “an” refer to one or more (i.e., at least one) of the

grammatical object of the article. By way of example, “an element” refers to one element or more than one elements, such as multiple elements.

[0059] The term “including” is used herein to mean the phrase “including but not limited to” and is used interchangeably with it.

[0060] The term “or” is used herein to mean the term “and/or” and is used interchangeably with it, unless the context clearly indicates otherwise.

[0061] As used herein, the terms “about” or “approximately,” when applied to one or more target values, refer to values similar to the stated reference value. In some embodiments, unless otherwise stated or clearly evident from the context, the terms “approximately” or “about” refer to a range of values that fall within 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater or less than) of the stated reference value (unless such a number would exceed 100% of the possible value).

[0062] As used herein, “HBV” refers to hepatitis B virus, including genotypes A, B, C, D, E, F, G, H, I, J, and their subtypes, and is not limited to any specific genotype.

[0063] “G,” “C,” “A,” and “U” each generally represent nucleotides containing guanine, cytosine, adenine, and uracil as their bases, respectively. “T” and “dT” are used interchangeably herein and refer to deoxyribonucleotides in which the nucleobase is thymine, such as deoxyribothymine, 2'-deoxythymidine, or thymidine. However, it should be understood that the terms “ribonucleotide,” “nucleotide,” or “deoxyribonucleotide” may also refer to a modified nucleotide (as further detailed below) or an alternative substituted moiety. Those skilled in the art will be well aware that guanine, cytosine, adenine, and uracil can be substituted by other moieties without substantially altering the base-pairing properties of an oligonucleotide (including a nucleotide containing such a substituted moiety). For example, but not limited to, a nucleotide containing inosine as its base can base-pair with nucleotides containing adenine, cytosine, or uracil. Thus, nucleotides containing uracil, guanine, or adenine may be substituted in the nucleotide sequences of the present disclosure by a nucleotide containing, for example, inosine. Sequences containing such substituted moieties are embodiments of the present disclosure.

[0064] The terms “RNAi agent” and “RNA interference agent” are used interchangeably

herein and refer to RNA agents as defined herein, which mediate targeted cleavage of RNA transcripts through the RNA-induced silencing complex (RISC) pathway. RNAi agents guide sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). RNAi agents regulate, for example, inhibit, the expression of HBV in cells, such as cells within a subject (e.g., a mammalian subject). RNAi molecules include single-stranded RNAi molecules, double-stranded siRNAs, and short hairpin RNAs (shRNAs).

[0065] The term “small interfering ribonucleic acid” or “siRNA” refers to small interfering ribonucleic acid RNAi molecules. It is a class of double-stranded RNA molecules, also known in the art as short interfering RNA or silencing RNA. siRNAs typically comprise a sense strand (also known as the passenger strand) and an antisense strand (also known as the guide strand), wherein each strand is 17 to 30 nucleotides in length, usually 19 to 25 nucleotides in length, wherein the antisense strand is complementary to the target nucleic acid (such as at least 95% complementary, such as fully complementary) (suitably a mature mRNA sequence), and the sense strand is complementary to the antisense strand, such that the sense and antisense strands form a duplex or duplex region. siRNA strands may form blunt-ended duplexes, or preferably, the 3' ends of the sense and antisense strands may form 3' overhangs, such as 1, 2, or 3 nucleotides, similar to the products produced by Dicer, which can form RISC substrates in vivo. Extended forms of Dicer substrates have been described in US 8349809 and US 8513207, which are incorporated herein by reference. In some embodiments, both the sense strand and the antisense strand have 3' overhangs of 2 nucleotides. Thus, the duplex region can be, for example, 17 to 25 nucleotides in length, such as 21 to 23 nucleotides in length.

[0066] The term “antisense strand” refers to the strand of RNAi (e.g., dsRNA) that includes a region substantially complementary to the target sequence. As used herein, the term “complementary region” refers to the region on the antisense strand that is substantially complementary to the sequence defined herein (e.g., the target sequence). When the complementary region is not fully complementary to the target sequence, mismatches can occur in the internal or terminal regions of the molecule. Typically, the most tolerated mismatches are in the terminal regions, for example, within 5, 4, 3, or 2 nucleotides at the 5' and/or 3' ends.

The term “sense strand” as used herein refers to the strand of RNAi that includes a region

substantially complementary to the region of the antisense strand (as defined herein).

[0067] The term “inhibition” and its variants as used herein may be used interchangeably with “reduction,” “silencing,” “downregulation,” “suppression,” and other similar terms, and includes any level of inhibition.

[0068] As used herein, the phrase “inhibition of HBV gene expression” and its variants include inhibition of the expression of HBV DNA, HBV mRNA, HBsAg, HBeAg, hepatitis B core antigen (HBcAg), and the like.

[0069] “Inhibition of HBV antigen expression” and its variants include inhibition of the expression of HBsAg, HBeAg, and HBcAg proteins.

[0070] “Inhibition of HBV gene expression” and its variants include any level of inhibition of HBV DNA, HBV mRNA, HBsAg, HBeAg, and HBcAg, such as at least partial inhibition of the expression of HBV DNA, HBV mRNA, HBsAg, HBeAg, and HBcAg, for example, inhibition of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

[0071] Based on any variable level associated with HBV gene expression, such as HBV DNA levels, HBV mRNA levels, HBV antigen protein levels, or HBV viral particle levels, the expression of HBV genes can be assessed. Inhibition can be assessed by a reduction in the absolute or relative levels of one or more of these variables compared to control levels. Control levels can be any type of control level utilized in the field, such as pre-treatment baseline levels or levels determined from similar untreated or control-treated (e.g., buffer-only control or inert agent control) subjects, cells, or samples.

[0072] As used herein, “patient” or “subject” is intended to include humans or non-human animals, preferably mammals, such as mice. Most preferably, the subject or patient is a human.

[0073] As used herein, “disease associated with HBV gene expression” is intended to include any disease associated with HBV genes or proteins. Such diseases may result, for example,

from excessive production of HBV antigen proteins, mutations in HBV genes, abnormal cleavage of HBV antigen proteins, or abnormal interactions between HBV antigen proteins and other proteins or endogenous or exogenous substances. Exemplary diseases associated with HBV gene expression include HBV infection-associated hepatitis, such as chronic hepatitis B, acute hepatitis B, as well as HBV/hepatitis D virus (HDV) co-infection and HBV/HIV co-infection-associated diseases.

[0074] As used herein, “therapeutically effective amount” is intended to include an amount of RNAi agent sufficient to achieve treatment of the disease (e.g., by mitigating, improving, or maintaining the existing disease or one or more disease symptoms) when administered to a patient for the treatment of a disease associated with HBV gene expression. The “therapeutically effective amount” may vary depending on the RNAi agent, how the agent is administered, the disease and its severity, medical history, age, weight, family history, genetic composition, the stage of the pathological process mediated by HBV expression, the type of prior or concomitant therapy (if any), and other individual characteristics of the patient to be treated.

[0075] As used herein, “prophylactically effective amount” is intended to include an amount of RNAi agent sufficient to prevent or ameliorate the disease or one or more symptoms of the disease when administered to a subject who has not yet experienced or shown symptoms of a disease associated with HBV gene expression but may be susceptible to it. To ameliorate the disease includes slowing the progression of the disease or reducing the severity of subsequent disease. The “prophylactically effective amount” may vary depending on the RNAi agent, how the agent is administered, the disease and its risk, medical history, age, weight, family history, genetic composition, the type of prior or concomitant therapy (if any), and other individual characteristics of the patient to be treated.

[0076] “Therapeutically effective amount” or “prophylactically effective amount” also includes an amount of RNAi agent that produces a desired local or systemic effect under a reasonable benefit/risk ratio applicable to any treatment. The RNAi agents used in the methods of the present disclosure may be administered in amounts sufficient to produce a reasonable benefit/risk ratio applicable to such treatments.

[0077] As used herein, the term “sample” includes fluids, cells, or tissues isolated from a

subject, as well as a collection of fluids, cells, or tissues present within the subject. Examples of biological fluids include blood, serum, serous fluid, plasma, cerebrospinal fluid, ocular fluid, lymph, urine, saliva, and the like. Tissue samples may include samples from tissues, organs, or localized regions. For example, samples may be derived from specific organs, parts of organs, or fluids or cells within these organs. In some embodiments, samples may be derived from the liver (e.g., the entire liver or certain segments of the liver, or certain types of cells within the liver, such as hepatocytes). In preferred embodiments, a “sample derived from a subject” refers to blood or plasma drawn from the subject. In other embodiments, a “sample derived from a subject” refers to liver tissue (or its subcomponents) derived from the subject.

[0078] In one aspect, the present disclosure provides a double-stranded RNAi agent, wherein the double-stranded RNAi agent comprises an antisense strand and a sense strand complementary to the antisense strand forming a duplex region, wherein the nucleotide sequence of the antisense strand is as shown in SEQ ID NO: 8, 11, or 13, or the nucleotide sequence of the antisense strand is a modified sequence of the sequence shown in SEQ ID NO: 8, 11, or 13.

[0079] In some embodiments, the double-stranded RNAi agent comprises an oligonucleotide duplex formed by pairing of the sense strand and the antisense strand; wherein the sense strand has a sequence as shown in SEQ ID NO: 1, 4, or 6, or a fragment thereof, or a modified sequence of the sequence or fragment thereof.

[0080] In some embodiments, the sense strand and the antisense strand comprise at least one modified nucleotide.

[0081] In some embodiments, the double-stranded RNAi agent has the function of inhibiting HBV gene expression.

[0082] In some embodiments, at least one of the modified nucleotides is selected from one or more of the group consisting of: deoxy-nucleotide, 3'-terminal deoxy-thymidine nucleotide, 2'-O-methyl-modified nucleotide, 2'-fluoro-modified nucleotide, 2'-deoxy-modified nucleotide, locked nucleotide, unlocked nucleotide, conformationally restricted nucleotide, constrained ethyl nucleotide, abasic nucleotide, 2'-amino-modified nucleotide, 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxy-modified nucleotide, 2'-O-methoxyethyl-modified nucleotide, 2'-O-alkyl-modified nucleotide, morpholino nucleotide,

phosphoramidate, nucleotide containing unnatural bases, tetrahydropyran-modified nucleotide, 1,5-anhydrohexitol-modified nucleotide, cyclohexenyl-modified nucleotide, nucleotide containing phosphorothioate groups, nucleotide containing methylphosphonate groups, nucleotide containing 5'-phosphate, and nucleotide containing 5'-phosphate mimetics.

[0083] In some embodiments, at least one strand of the double-stranded RNAi agent comprises a 3' overhang of at least 2 nucleotides.

[0084] In some embodiments, the duplex region of the double-stranded RNAi agent is 20 nucleotide pairs in length.

[0085] In some embodiments, the sense strand of the double-stranded RNAi agent has 20 nucleotides, and the antisense strand has 22 nucleotides.

[0086] In some embodiments, all modifications on the nucleotides of the sense and antisense strands are chemical modifications at the 2' position of the nucleotide ribose.

[0087] In some embodiments, the chemical modifications at the 2' position of the nucleotide ribose are selected from one or more of the group consisting of: 2'-methoxy, 2'-O-methoxyethyl, 2'-fluoro, 2'-benzyloxy, 2'-methylcarbonylamino, and 2'-pyridylmethoxy.

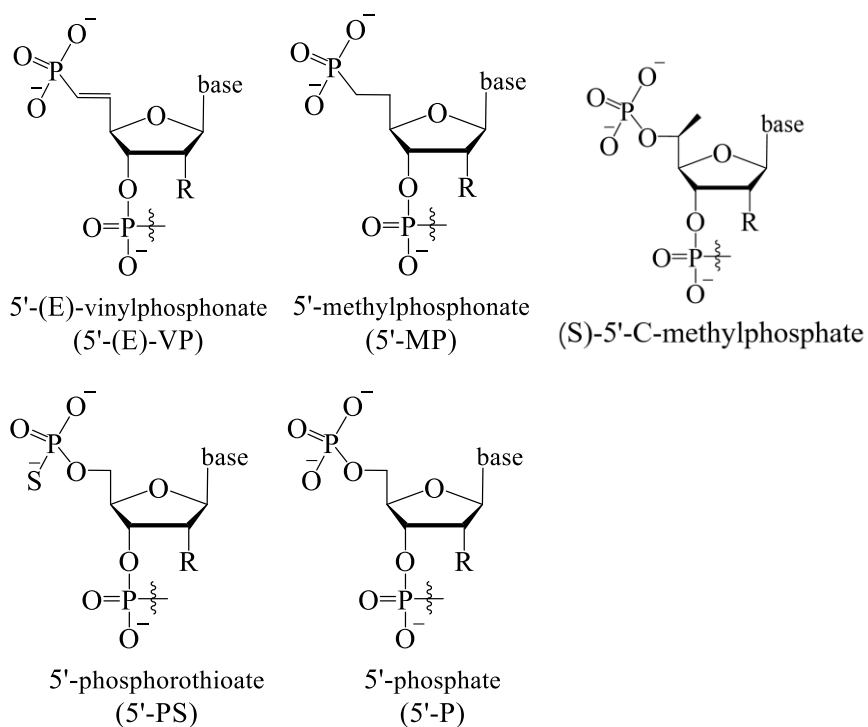
[0088] In some embodiments, the chemical modification at the 2' position of the nucleotide ribose is 2'-methoxy or 2'-fluoro.

[0089] In some embodiments, the nucleotides are linked by 3',5'-phosphodiester bonds.

[0090] In some embodiments, the 3',5'-phosphodiester bond comprises a phosphorothioate modification.

[0091] In some embodiments, a 5' carbon atom of the 5' terminal nucleotide glycoside of the antisense strand is phosphorylated.

[0092] In some embodiments, the phosphorylated 5' phosphorylated group includes one or more selected from: 5'-vinylphosphonate group, 5'-methylphosphonate group, 5'-C-methylphosphate group, 5'-phosphorothioate group, and 5'-phosphate group, with the structure being;



[0093] R is hydrogen, hydroxyl, amino, C₁₋₄ alkyl, aryl, C₁₋₄ alkoxy, C₁₋₄ alkylcarbonylamino, or halogen;

[0094] the base is selected from any one of adenine, guanine, cytosine, thymine, and uracil.

[0095] In some embodiments, terminal nucleotides of the sequence are linked by a 3',5'-phosphodiester bond containing a phosphorothioate modification, forming a chirally pure 3',5'-phosphorothioate bond.

[0096] In some embodiments, a 5' end of the sense strand and the antisense strand comprises 1 to 3 phosphorothioate linkages, and a 3' end of the antisense strand comprises 1 to 3 phosphorothioate linkages.

[0097] In some embodiments, the antisense strand adopts one of the modification patterns outlined in the table below:

N o .	Antisense strand (AS) 5'-3'																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
A	2'- - O M e +	2'- - F + P S	2'- - O M e	2'- - F	2'- - F	2'- - F	2'- - O M e	2'- - O M e	2'- - O M e	2'- - O M e	2'- - O M e	2'- - O M e	2'- - O M e	2'- - F	2'- - O M e	2'- - F	2'- - O M e	2'- - O M e	2'- - O M e	2'- - O Me +P S	2'- - O M e +	2'- - O M e

	P S + E V P																			P S		
B	2' - O M e + P S + E V P	2' - F + P S	2' - F	2' - F	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2'- O Me +P S	2' - O M e + P S	2' - O M e
C	2' - O M e + P S + E V P	2' - F + P S	2' - O M e	2' - O M e	2' - F	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2'- O Me +P S	2' - O M e + P S	2' - O M e
D	2' - O M e + P S + E V P	2' - F + P S	2' - F	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2'- O Me +P S	2' - O M e + P S	2' - O M e
E	2' -	2' -	2' -	2' -	2' -	2' -	2' -	2' -	2' -	2' -	2' -	2' -	2' -	2' -	2' -	2' -	2' -	2' -	2' -	2'- O	2' -	2' -

	O M e + P S + E V P	F + P S	O M e	O M e	O M e	- F	O M e	O M e	O M e	O M e	O M e	O M e	O M e	- F	O M e	- F	O M e	O M e	O M e	Me +P S	O M e + P S	O M e
F	2' - O M e + P S + E V P	2' - F + P S	2' - F + P S	2' - O M e	2' - F	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2'- O Me +P S	2' - O M e + P S	2' - O M e

;

or

N o .	Antisense strand (AS) 5'-3'																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
A	2'- O Me + P S + E V P	2'- - F + P S	2'- O Me	2'- F	2'- - F	2'- - F	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - F	2'- - O Me	2'- - F	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- O Me +P S	2'- - O Me + P S	2'- - O Me
B	2'- O M	2'- - F	2'- F	2'- F	2'- - O M	2'- - F	2'- - O M	2'- - O M	2'- - O M	2'- - O M	2'- - O M	2'- - O M	2'- - O M	2'- - F	2'- - O M	2'- - F	2'- - O M	2'- - O M	2'- - O M	2'- - O M	2'- O Me +P	2'- - O M	2'- - O M

	e + P S + E V P	+ P S			e		e	e	e	e	e	e	e		e		e	e	e	e	S	e + P S	e
C	2' - O M e + P S + E V P	2' - F + P S	2' - O M e	2' - O M e	2' - F	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e + P S	2' - O M e + P S	2' - O M e
D	2' - O M e + P S + E V P	2' - F + P S	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e + P S	2' - O M e + P S	2' - O M e
E	2' - O M e + P S + E V P	2' - F + P S	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e + P S	2' - O M e + P S	2' - O M e

F	2'-O-Me + P-S + E-V-P	2'-F + P-S	2'-O-Me	2'-F	2'-F	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-F	2'-O-Me	2'-F	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me + P-S	2'-O-Me + P-S	2'-O-Me
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[0098] and/or, the sense strand adopts one of the modification patterns outlined in the table below:

N o .	Sense strand (SS) 5'-3'																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
a	2'-O-Me + P-S	2'-O-Me + P-S	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me
b	2'-O-Me + P-S	2'-O-Me + P-S	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me	2'-O-Me

;

N o .	Sense strand (SS) 5'-3'																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
a	2'- O M e + P	2'- O M e + P	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2 ' - F	2'- O M e	2 ' - F	2'- F	2 ' - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2 ' - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e

	S	S																			
b	2'- O M e + P S	2'- O M e + P S	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2 ' - F	2'- O M e	2 ' - F	2'- O M e	2 ' - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2 ' - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e

[0099] wherein 2'-OMe is 2'-methoxy; 2'-F is 2'-fluoro; PS is phosphorothioate backbone; EVP is 5'-(E)-vinylphosphonate.

[0100] In some embodiments, the modification patterns of the double-stranded RNAi agent are as follows:

[0101] the siRNA modification template with antisense strand modified by pattern A and sense strand modified by pattern a is designated as DV27P;

[0102] the siRNA modification template with antisense strand modified by pattern A and sense strand modified by pattern b is designated as DV29P;

[0103] the siRNA modification template with antisense strand modified by pattern B and sense strand modified by pattern a is designated as DV26P;

[0104] the siRNA modification template with antisense strand modified by pattern B and sense strand modified by pattern b is designated as DV28P;

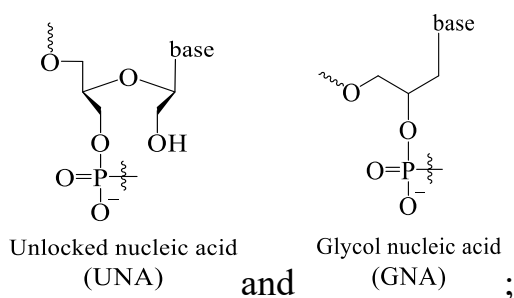
[0105] the siRNA modification template with antisense strand modified by pattern C and sense strand modified by pattern b is designated as DV32P;

[0106] the siRNA modification template with antisense strand modified by pattern D and sense strand modified by pattern b is designated as DV34P;

[0107] the siRNA modification template with antisense strand modified by pattern E and sense strand modified by pattern a is designated as DV25P; or

[0108] the siRNA modification template with antisense strand modified by pattern F and sense strand modified by pattern b is designated as DV33P.

[0109] In some embodiments, the antisense strand is modified with modifying groups from the second to the eighth positions starting from the 5' end, wherein the modifying groups are selected from one or more of UNA, GNA, and DNA, and the structures of UNA and GNA are



[0110] the base is selected from any one of adenine, guanine, cytosine, thymine, and uracil.

[0111] In some embodiments, the double-stranded RNAi agent comprises an oligonucleotide duplex selected from any one of the following sense and antisense strand pairs:

[0112] (1) the sense strand has the sequence as shown in SEQ ID NO: 81, and the antisense strand has the sequence as shown in SEQ ID NO: 179, 180, 181, 182, or 184;

[0113] (2) the sense strand has the sequence as shown in SEQ ID NO: 82, and the antisense strand has the sequence as shown in SEQ ID NO: 179, 180, or 183;

[0114] (3) the sense strand has the sequence as shown in SEQ ID NO: 83, and the antisense strand has the sequence as shown in SEQ ID NO: 185 or 186;

[0115] (4) the sense strand has the sequence as shown in SEQ ID NO: 84, and the antisense strand has the sequence as shown in SEQ ID NO: 186;

[0116] (5) the sense strand has the sequence as shown in SEQ ID NO: 85, and the antisense strand has the sequence as shown in SEQ ID NO: 186;

[0117] (6) the sense strand has the sequence as shown in SEQ ID NO: 96, and the antisense strand has the sequence as shown in SEQ ID NO: 201, 202, 203, or 204;

[0118] (7) the sense strand has the sequence as shown in SEQ ID NO: 97, and the antisense strand has the sequence as shown in SEQ ID NO: 201 or 202;

[0119] (8) the sense strand has the sequence as shown in SEQ ID NO: 98, and the antisense strand has the sequence as shown in SEQ ID NO: 205 or 206;

[0120] (9) the sense strand has the sequence as shown in SEQ ID NO: 99, and the antisense strand has the sequence as shown in SEQ ID NO: 206;

[0121] (10) the sense strand has the sequence as shown in SEQ ID NO: 100, and the antisense strand has the sequence as shown in SEQ ID NO: 206;

[0122] (11) the sense strand has the sequence as shown in SEQ ID NO: 106, and the antisense

strand has the sequence as shown in SEQ ID NO: 213, 214, 215, or 216;

[0123] (12) the sense strand has the sequence as shown in SEQ ID NO: 107, and the antisense strand has the sequence as shown in SEQ ID NO: 214 or 213;

[0124] (13) the sense strand has the sequence as shown in SEQ ID NO: 108, and the antisense strand has the sequence as shown in SEQ ID NO: 217 or 218;

[0125] (14) the sense strand has the sequence as shown in SEQ ID NO: 109, and the antisense strand has the sequence as shown in SEQ ID NO: 218;

[0126] (15) the sense strand has the sequence as shown in SEQ ID NO: 110, and the antisense strand has the sequence as shown in SEQ ID NO: 218;

[0127] (16) the sense strand has the sequence as shown in SEQ ID NO: 306, and the antisense strand has the sequence as shown in SEQ ID NO: 321.

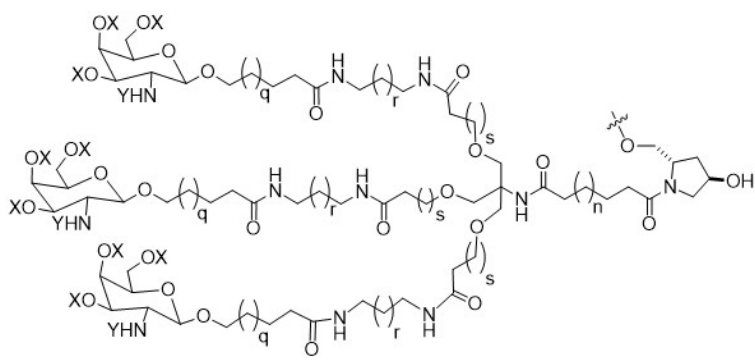
[0128] The double-stranded RNA (dsRNA) agent (double-stranded RNAi agent) of the present disclosure may optionally be conjugated to one or more ligands. The ligand may be attached to the sense strand, the antisense strand, or both strands at the 3' end, the 5' end, or both ends. For example, the ligand may be conjugated to the sense strand. In a preferred embodiment, the ligand is attached to the 3' end of the sense strand. In one preferred embodiment, the ligand is a GalNAc ligand.

[0129] In another aspect, the present disclosure provides a conjugate comprising the aforementioned double-stranded RNAi agent and a ligand conjugated to the double-stranded RNAi agent.

[0130] In some embodiments, the ligand is conjugated to a 3'-end or a 5'-end of an oligonucleotide sense strand.

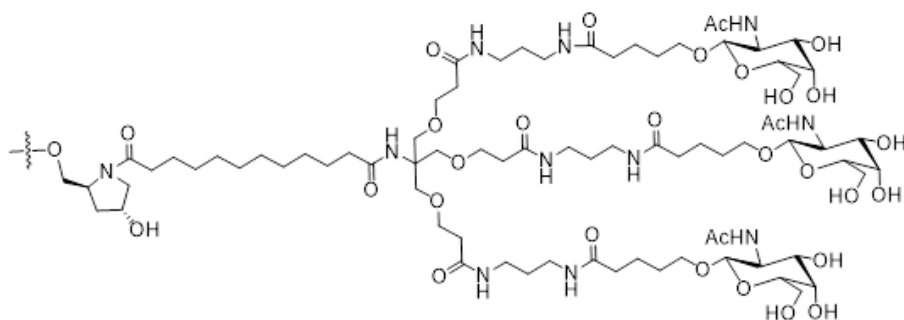
[0131] In some embodiments, the ligand is one or more GalNAc derivatives attached via a bivalent or trivalent branched linker, or a GalNAc derivative attached via a monovalent linker.

[0132] In some embodiments, the ligand is

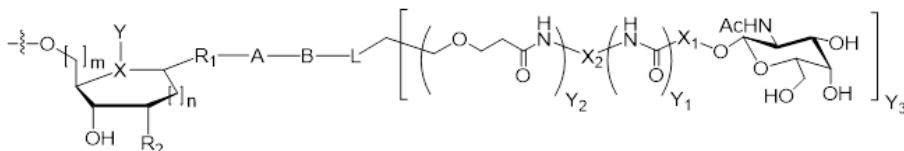


[0133] wherein X is hydrogen, a hydroxyl protecting group, or H, and the hydroxyl protecting group includes acetyl, benzoyl, or isobutyryl; Y is an amine protecting group or H, and the amine protecting group is formyl, acetyl, propionyl, n-butyryl, or isobutyryl; n is an integer between 0 and 20; q, r, and s are independently integers between 1 and 7.

[0134] In some embodiments, the ligand is



[0135] In some embodiments, the ligand is



[0136] wherein X is oxygen, nitrogen, or sulfur;

[0137] Y is an alkyl or aromatic group;

[0138] R₁ is oxygen or sulfur;

[0139] R₂ is hydrogen, an amino group, a C₁₋₄ alkyl group, an aromatic group, a C₁₋₄ alkoxy group, or a halogen;

[0140] A is -(CH₂)_a-, -(CH₂CH₂O)_b-, -((CH₂)_cNHCO)_d-, or -((CH₂)_cCONH)_d-, where a is an integer from 1 to 15, b is an integer from 1 to 7, c is an integer from 1 to 7, and d is an integer from 1 to 5;

[0141] B is -(CH₂)_e-, where e is an integer from 0 to 7;

[0142] L is -CONH- or -NHCO-;

[0143] X_1 is $-(CH_2)_f-$ or $-(CH_2CH_2O)_fCH_2-$, where f is an integer from 1 to 5;

[0144] X_2 is $-(CH_2)_g-$, where g is an integer from 1 to 6;

[0145] Y_1 is 0 or 1;

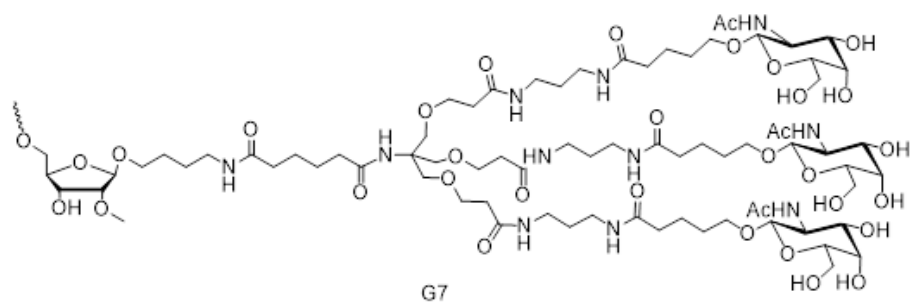
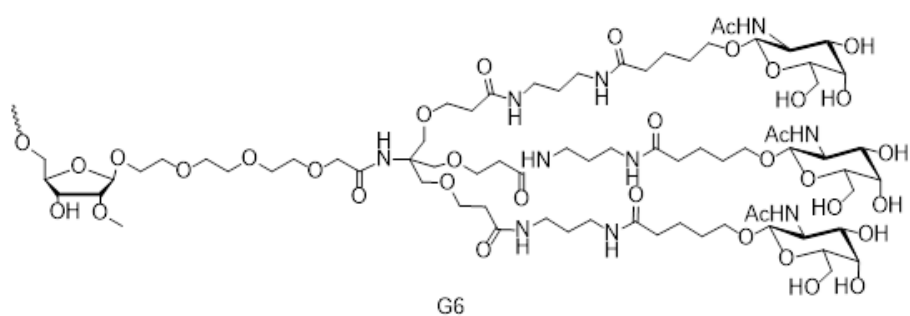
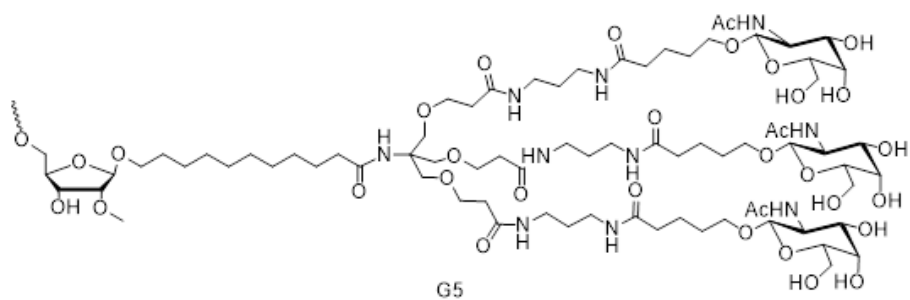
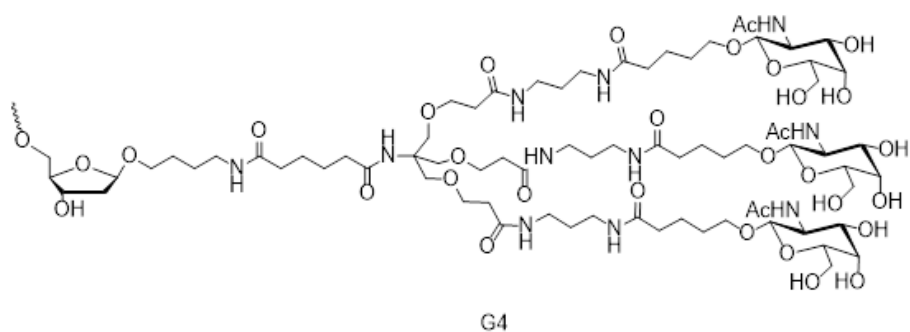
[0146] Y_2 is 0, 1, or 2;

[0147] Y_3 is 1, 2, or 3;

[0148] m is an integer from 0 to 4;

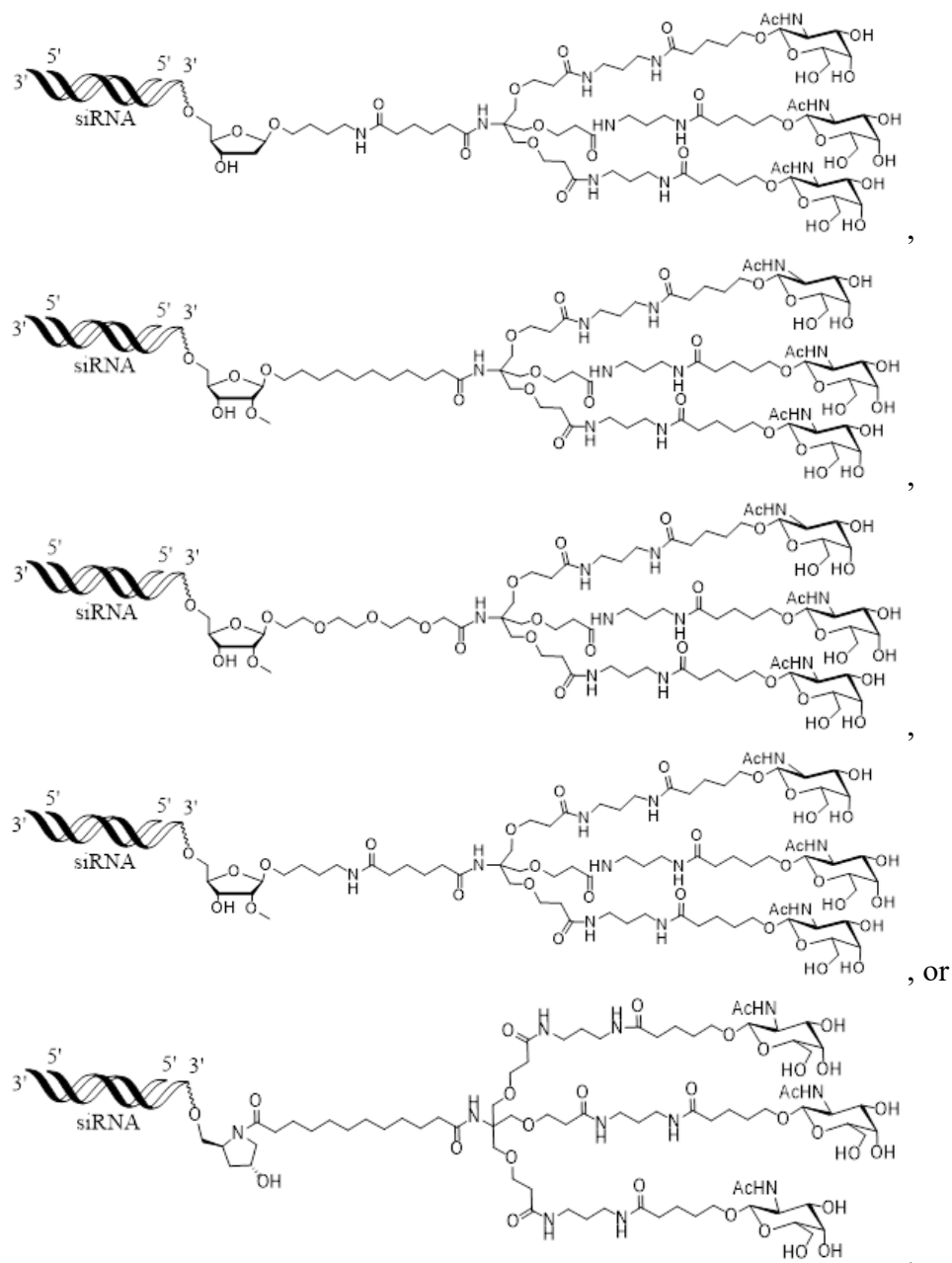
[0149] n is an integer from 0 to 4.

[0150] In some embodiments, the ligand is G4, G5, G6, or G7:

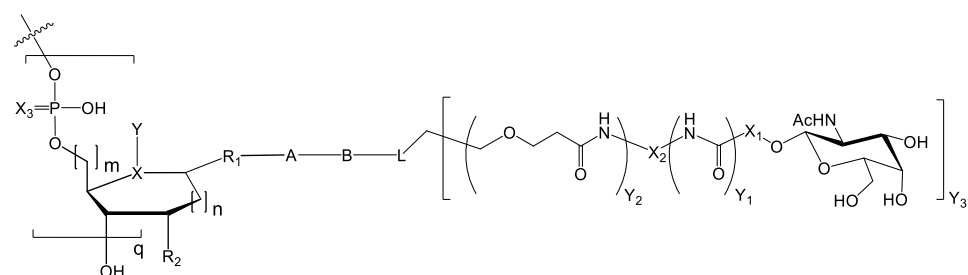


, or

[0151] In some embodiments, the ligand is represented by the following structures:

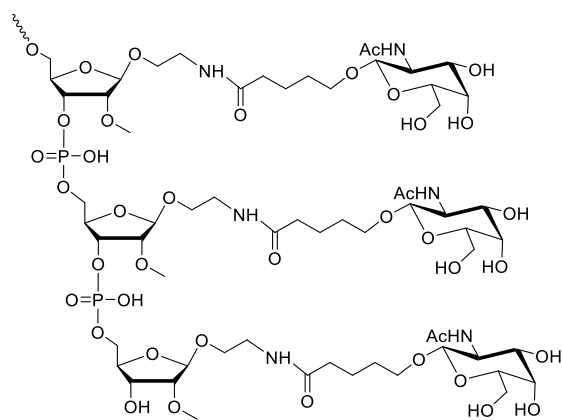


[0152] In some embodiments, the ligand is:

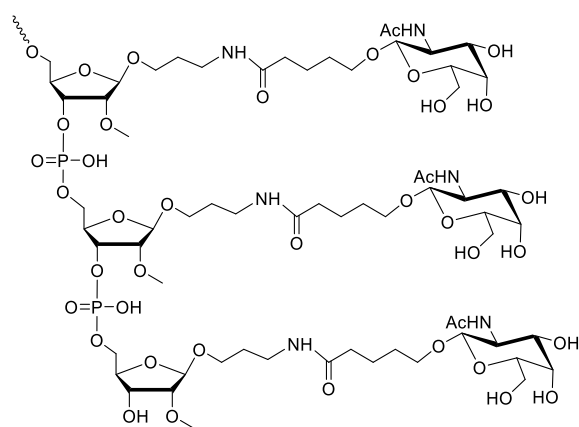


[0153] wherein X is oxygen, nitrogen, or sulfur;

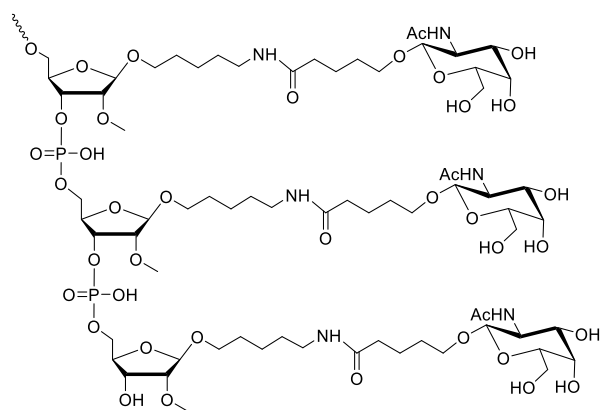
[0154] Y is an alkyl or aromatic group;



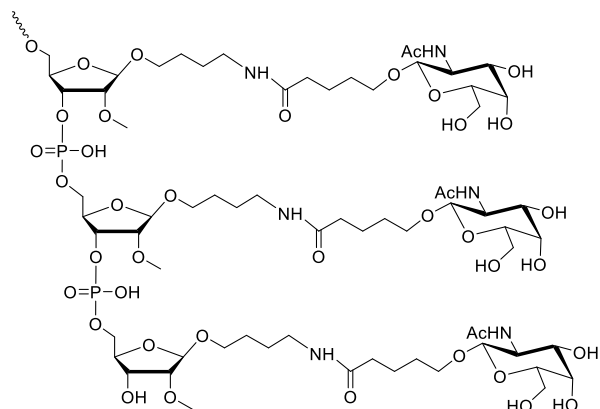
G102



G103

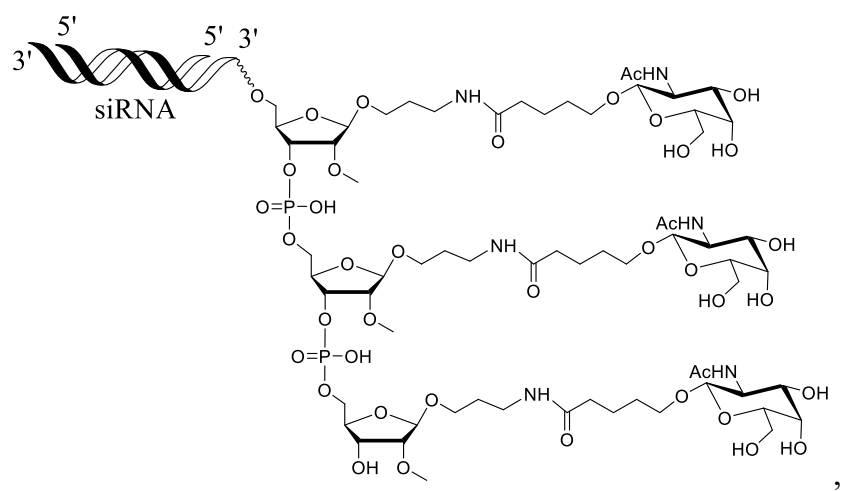
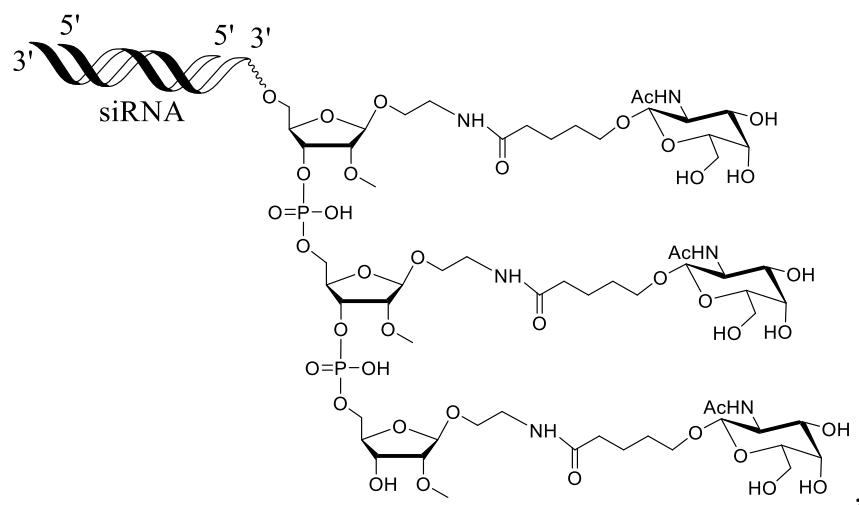
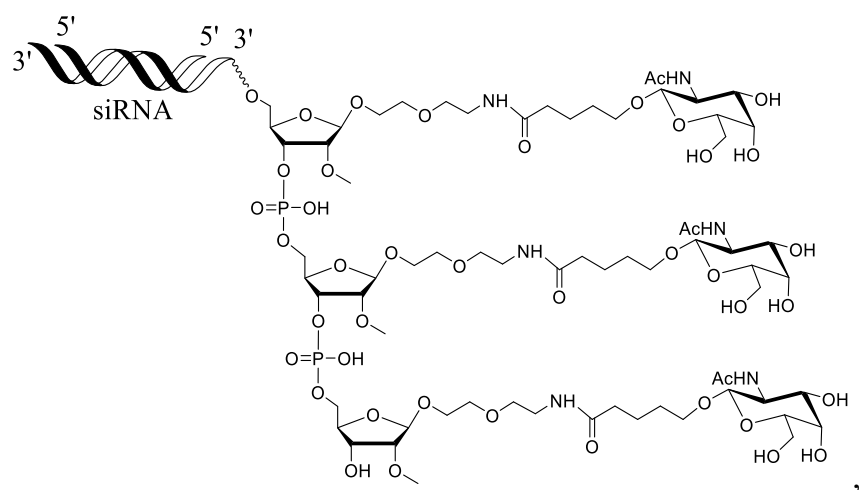


G105



G106

[0168] In some embodiments, the ligand is represented by the following structures:



expression.

[0175] In another aspect, the present disclosure provides a pharmaceutical composition comprising the aforementioned double-stranded RNAi agent or the aforementioned conjugate, along with a pharmaceutically acceptable carrier.

[0176] In one embodiment, the present disclosure provides a pharmaceutical composition comprising the double-stranded RNAi agent as described herein and a pharmaceutically acceptable carrier. The pharmaceutical composition comprising the double-stranded RNAi agent can be used to treat diseases or disorders associated with the expression or activity of HBV genes, such as chronic hepatitis B. Such pharmaceutical compositions are formulated based on delivery models. One example is a composition formulated for systemic administration via parenteral delivery, such as intravenous (IV) delivery. Another example is a composition formulated for direct delivery to the brain parenchyma, such as through infusion into the brain, for example, via continuous pump infusion.

[0177] The pharmaceutical composition comprising the double-stranded RNAi agent of the present disclosure may be, for example, a solution with or without a buffer or a composition comprising a pharmaceutically acceptable carrier. Such compositions include, for example, aqueous or crystalline compositions, liposome formulations, micelle formulations, emulsions, and gene therapy vectors.

[0178] In the method of the present disclosure, the double-stranded RNAi agent may be administered in a solution. A free double-stranded RNAi agent may be administered in a non-buffered solution, such as in normal saline or in water. Alternatively, the free siRNA may also be administered in a suitable buffered solution. The buffered solution may include acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In a preferred embodiment, the buffered solution is phosphate-buffered saline (PBS). The pH and osmolality of the buffered solution comprising the double-stranded RNAi agent may be adjusted to make it suitable for administration to a subject.

[0179] In some embodiments, the buffered solution further comprises an agent for controlling the osmolality of the solution, such that the osmolality is maintained at a desired value, for example, at the physiological value of human plasma. Solutes that may be added to the buffered solution to control osmolality include, but are not limited to, proteins, peptides, amino

acids, non-metabolic polymers, vitamins, ions, sugars, metabolites, organic acids, lipids, or salts. In some embodiments, the agent for controlling the osmolality of the solution is a salt. In some embodiments, the agent for controlling the osmolality of the solution is sodium chloride or potassium chloride.

[0180] The pharmaceutical composition of the present disclosure may be administered at a dose sufficient to inhibit the expression of HBV genes. Typically, a suitable dose of the double-stranded RNAi agent of the present disclosure ranges from about 0.001 to 200.0 mg per kilogram of body weight per day, and usually ranges from about 0.1 to 50 mg per kilogram of body weight per day. For example, the double-stranded RNAi agent (e.g., dsRNA) may be administered at a single dose of about 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 31, 32, 33, 34, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg.

[0181] The pharmaceutical composition may be administered once daily, or the double-stranded RNAi agent may be administered in two, three, or more sub-doses at appropriate intervals throughout the day. Alternatively, it may be administered via continuous infusion or delivery using a controlled-release formulation. In such cases, the amount of double-stranded RNAi agent contained in each sub-dose must be proportionally reduced to achieve the total daily dose. Dose units may also be formulated for delivery over several days, for example, using conventional sustained-release formulations that provide prolonged release of the double-stranded RNAi agent over a period of days. Sustained-release formulations are well-known in the art and are particularly useful for delivering agents to specific sites, and thus they can be used with the agents of the present disclosure. In this embodiment, the dose unit comprises a corresponding multiple of the daily dose.

[0182] In other embodiments, a single dose of the pharmaceutical composition may last for

an extended period, such that subsequent doses are administered at intervals of no more than 3, 4, or 5 days, or at intervals of no more than 1, 2, 3, or 4 weeks. In some embodiments of the present disclosure, a single dose of the pharmaceutical composition is administered once weekly. In other embodiments of the present disclosure, a single dose of the pharmaceutical composition is administered once monthly.

[0183] Those skilled in the art will understand that certain factors may influence the dose and timing required to effectively treat a subject, and these factors include, but are not limited to, the severity of the disease or disorder, prior treatments, the general health and/or age of the subject, and the presence of other diseases. Furthermore, treating a subject with a therapeutically effective dose of the composition may involve a single treatment or a series of treatments. As described elsewhere herein, the effective dose and in vivo half-life of the various double-stranded RNAi agents encompassed by the present disclosure can be estimated using conventional methods or in vivo testing based on suitable animal models.

[0184] Depending on whether local or systemic treatment is desired and depending on the area to be treated, the pharmaceutical composition of the present disclosure may be administered in various ways. Administration may be topical (e.g., via a skin patch); pulmonary, for example, via inhalation or insufflation of a powder or aerosol, including through a nebulizer; intratracheal; intranasal; epidermal and transdermal; oral; or parenteral. Parenteral administration includes intravenous, intra-arterial, subcutaneous, intraperitoneal, or intramuscular injection or infusion; subdermal, for example, via an implantable device; or intracranial, such as intraparenchymal, intrathecal, or intraventricular administration.

[0185] The double-stranded RNAi agent used in the composition and method of the present disclosure may be formulated for delivery within membranous molecular assemblies, such as liposomes or micelles. As used herein, the term "liposome" refers to a vesicle composed of amphipathic lipids arranged in at least one bilayer (e.g., a single bilayer or multiple bilayers). Liposomes include unilamellar or multilamellar vesicles with a membrane formed from lipophilic materials and an aqueous interior. The aqueous portion comprises the composition comprising the double-stranded RNAi agent. The lipophilic material separates the aqueous interior from an aqueous exterior, which typically does not comprise the composition comprising the double-stranded RNAi agent (although in some instances, it may). Liposomes

are useful for transferring and delivering active ingredients to sites of action. Because the liposome membrane is structurally similar to biological membranes, when liposomes are administered to a tissue, the liposome bilayer fuses with the cell membrane. As the liposome fuses with the cell, the internal aqueous contents, comprising the double-stranded RNAi agent, are delivered into the cell, where the double-stranded RNAi agent can specifically bind to a target RNA and mediate RNA interference (RNAi). In some cases, these liposomes are also specifically targeted, for example, to direct the double-stranded RNAi agent to a specific cell type.

[0186] Liposomes comprising the double-stranded RNAi agent may be prepared using various methods. In one example, lipid components of the liposome are dissolved in a detergent, allowing the formation of micelles with the lipid components. For example, the lipid components may include amphipathic cationic lipids or lipid conjugates. The detergent may have a high critical micelle concentration (CMC) and can be non-ionic. Exemplary detergents include cholate, CHAPS, octyl glucoside, deoxycholate, and lauroyl sarcosinate. Subsequently, the double-stranded RNAi agent is added to the micelles formed with the lipid components. The cationic groups on the lipids interact with the double-stranded RNAi agent and condense around it to form a liposome. After condensation, the detergent is removed, for example, through dialysis, to obtain a liposomal formulation of the double-stranded RNAi agent.

[0187] Double-stranded RNAi agents, such as the dsRNA of the present disclosure, may be fully encapsulated in lipid formulations, such as lipid nanoparticles (LNP) or other nucleic acid-lipid particles.

[0188] As used herein, the term "lipid nanoparticle (LNP)" refers to a stable nucleic acid-lipid particle. A LNP contains a cationic lipid, a non-cationic lipid, and a lipid that prevents particle aggregation (e.g., a PEG-lipid conjugate). LNPs are highly useful for synthetic applications because they exhibit prolonged circulation lifetimes after intravenous (i.v.) injection and accumulate at distal sites (e.g., sites physically separated from the administration site).

[0189] In one embodiment, the lipid-to-drug ratio (mass/mass ratio) (e.g., the lipid-to-dsRNA ratio) will range from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or from about 6:1 to

about 9:1.

[0190] In some embodiments, the double-stranded RNAi agent or the conjugate is administered in a non-buffered solution.

[0191] In some embodiments, the non-buffered solution is saline or water.

[0192] In some embodiments, the double-stranded RNAi agent or the conjugate is administered with a buffered solution.

[0193] In some embodiments, the buffered solution includes acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof.

[0194] In some embodiments, the buffered solution is phosphate-buffered solution.

[0195] In some embodiments, the double-stranded RNAi agent or the conjugate is formulated into a lipid formulation for delivery within membranous molecular assemblies.

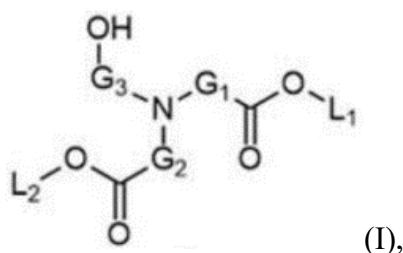
[0196] In some embodiments, the lipid formulation is a nucleic acid-lipid particle.

[0197] In some embodiments, the lipid formulation is a lipid nanoparticle.

[0198] In some embodiments, the mass-to-mass ratio of lipid to the double-stranded RNAi agent or the conjugate is 1:1 to 50:1, 1:1 to 25:1, 3:1 to 15:1, 4:1 to 10:1, 5:1 to 9:1, or 6:1 to 9:1.

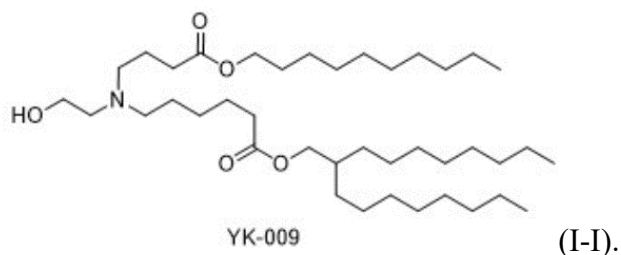
[0199] In some embodiments, the lipid nanoparticle comprises a cationic lipid, a neutral lipid, a structural lipid, and a polymer-conjugated lipid.

[0200] In some embodiments, the cationic lipid is a compound of formula (I), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,

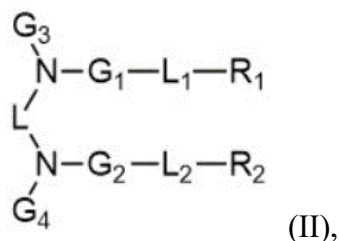


[0201] wherein G₁ is a C₁₋₆ alkylene group; G₂ is a C₂₋₈ alkylene group; G₃ is a C₁₋₃ alkylene group; L₁ is a C₆₋₁₅ linear alkyl group; L₂ is a C₁₂₋₂₅ branched alkyl group.

[0202] In some embodiments, the cationic lipid is YK-009 of formula (I-I) (see CN114044741B):

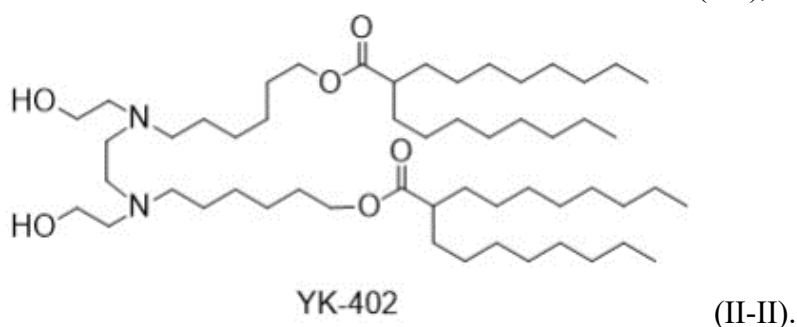
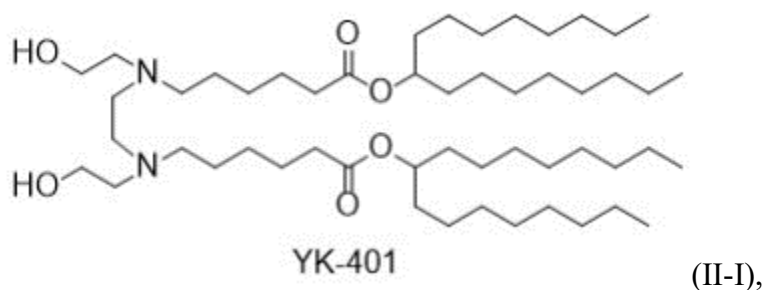


[0203] In some embodiments, the cationic lipid is a compound of formula (II), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,

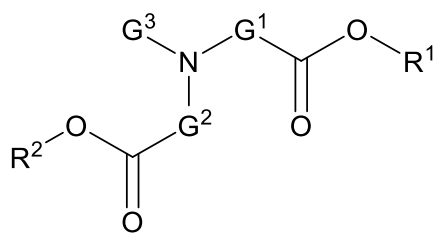


[0204] wherein G_1 is a C_{2-8} alkylene group; G_2 is a C_{2-8} alkylene group; L_1 is $-C(O)O-$ or $-OC(O)-$; L_2 is $-C(O)O-$ or $-OC(O)-$; R_1 is a C_{6-25} linear or branched alkyl group; R_2 is a C_{6-25} linear or branched alkyl group; G_3 is $HO(CH_2)_2-$ or $HO(CH_2)_3-$; G_4 is $HO(CH_2)_2-$ or $HO(CH_2)_3-$; L is $-(CH_2)_2-$, $-(CH_2)_3-$, or $-(CH_2)_4-$.

[0205] In some embodiments, the cationic lipid is YK-401 of formula (II-I) or YK-402 of formula (II-II) (see CN115784921B):



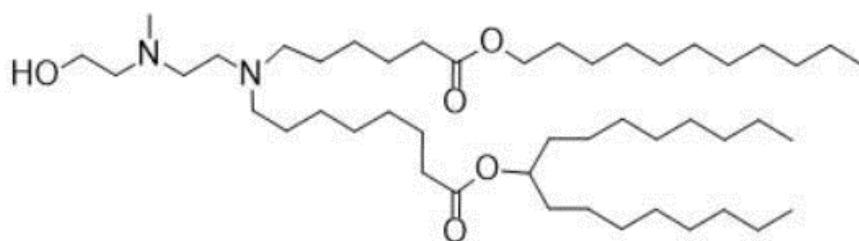
[0206] In some embodiments, the cationic lipid is a compound of formula (III), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,



(III),

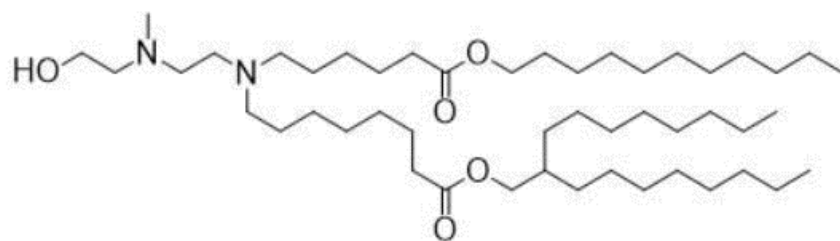
[0207] wherein G_1 is a C_{1-6} alkylene group; G_2 is a C_{2-8} alkylene group; R_1 is a C_{6-20} linear or branched alkyl group; R_2 is a C_{12-25} branched alkyl group; G_3 is $HO(CH_2)_2N(CH_3)(CH_2)_2-$, $HO(CH_2)_2N(CH_2CH_3)(CH_2)_2-$, $(HO(CH_2)_2)_2N(CH_2)_2-$, $CH_3O(CH_2)_2N(CH_3)(CH_2)_2-$, $(CH_3)_2N(CH_2)_3SC(O)O(CH_2)_2-$, $(CH_3)_2N(CH_2)_3SC(O)-$, $CH_3NH(CH_2)_2N(CH_3)(CH_2)_2-$, or $CH_3CH_2NH(CH_2)_2-$.

[0208] In some embodiments, the cationic lipid is YK-201 of formula (III-I) or YK-202 of formula (III-II) (see CN115677518B):



YK-201

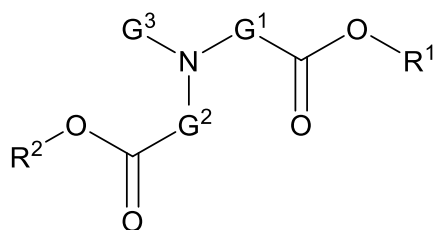
(III-I),



YK-202

(III-II).

[0209] In some embodiments, the cationic lipid is a compound of formula (IV), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,

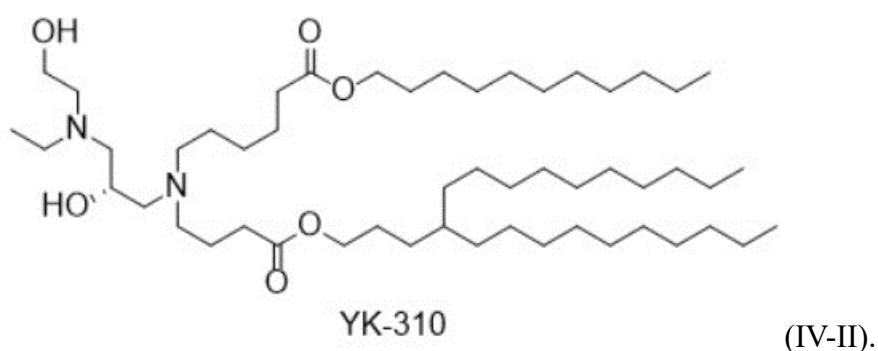
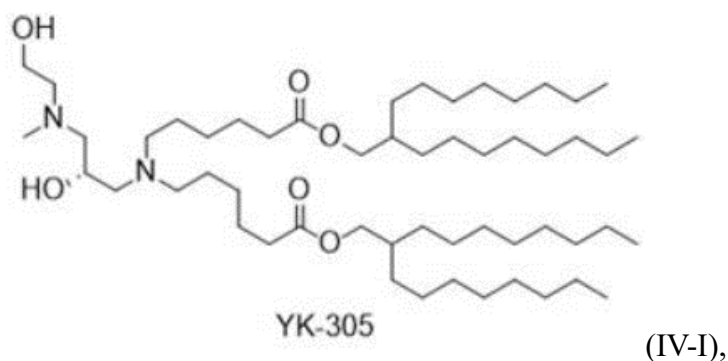


(IV),

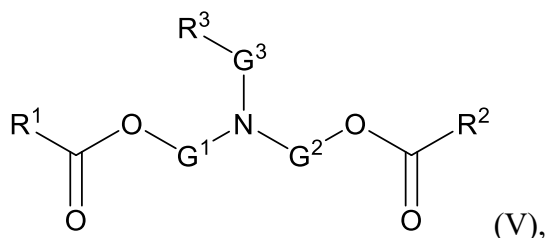
[0210] wherein G_1 is a C_{1-8} alkylene group; G_2 is a C_{2-8} alkylene group; R_1 is a C_{6-25} linear or branched alkyl group; R_2 is a C_{12-25} linear or branched alkyl group; G_3 is

$\text{HO}(\text{CH}_2)_2\text{N}(\text{R}_3)\text{CH}_2\text{CH}(\text{OH})\text{CH}_2-$, where R_3 is $-\text{CH}_3$, $-\text{CH}_2\text{CH}_3$, or $-\text{CH}_2\text{CH}_2\text{OH}$.

[0211] In some embodiments, the cationic lipid is YK-305 of formula (IV-I) or YK-310 of formula (IV-II) (see CN115745820B):

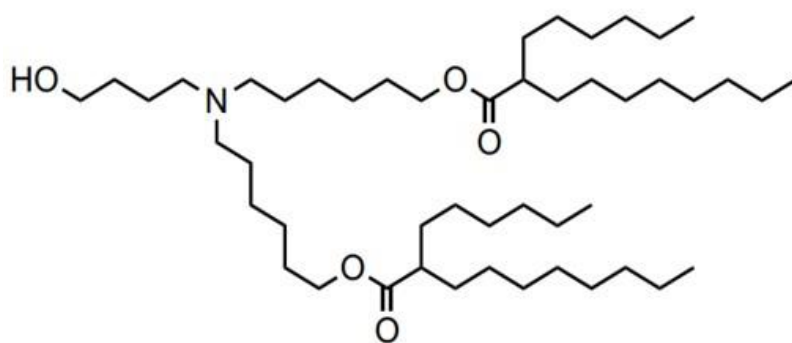


[0212] In some embodiments, the cationic lipid is a compound of formula (V), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,



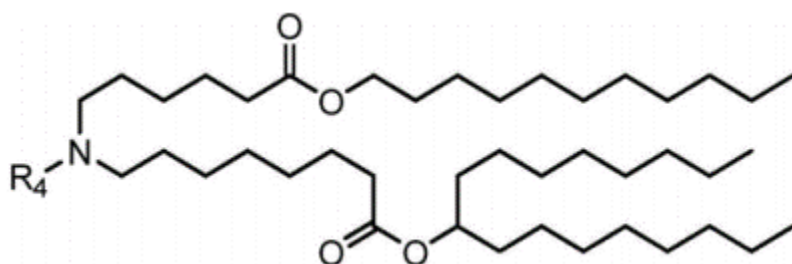
[0213] G^1 and G^2 are each independently an unsubstituted C_6 – C_{10} alkylene group; G^3 is an unsubstituted C_1 – C_{12} alkylene group; R^1 and R^2 are each independently a C_6 – C_{24} alkyl group or a C_6 – C_{24} alkenyl group; R^3 is OR^5 , N , $-\text{C}(=\text{O})\text{OR}^4$, $-\text{OC}(=\text{O})\text{R}^4$, or $-\text{NR}^5\text{C}(=\text{O})\text{R}^4$; R^4 is a C_1 – C_{12} hydrocarbon group; and R^5 is H or a C_1 – C_6 hydrocarbon group.

[0214] In some embodiments, the cationic lipid is ALC0315 of formula (V-I) (see CN108368028B):



(V-I).

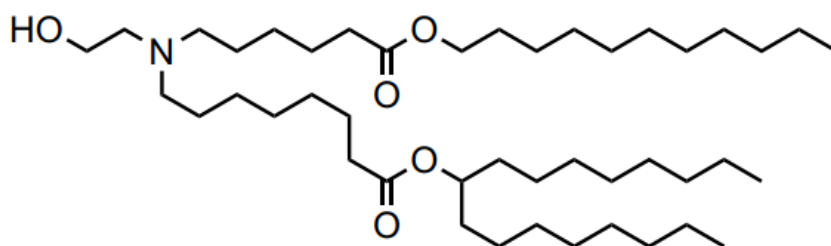
[0215] In some embodiments, the cationic lipid is a compound of formula (VI), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,



(VI),

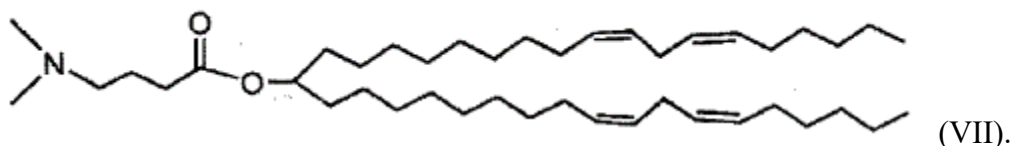
[0216] R₄ is selected from -(CH₂)_nQ and -(CH₂)_nCHQR; Q is selected from the group consisting of -OR, -OH, -O(CH₂)_nN(R)₂, -OC(O)R, -CX₃, -CN, -N(R)C(O)R, -N(H)C(O)R, -N(R)S(O)₂R, -N(H)S(O)₂R, -N(R)C(O)N(R)₂, -N(H)C(O)N(R)₂, -N(H)C(O)N(H)(R), -N(R)C(S)N(R)₂, -N(H)C(S)N(R)₂, -N(H)C(S)N(H)(R), -N(R)S(O)₂R₈, and a heterocyclic group; n is 1, 2, or 3.

[0217] In some embodiments, the cationic lipid is SM102 of formula (VI-I) (see CN110520409A):



(VI-I).

[0218] In some embodiments, the cationic lipid is the compound DLIN-MC3-DMA of formula (VII) (see CN102625696B), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,



[0219] In some embodiments, the cationic lipid comprises one or more selected from YK-009, YK-401, YK-305, ALC-0315, SM-102, and DLin-MC3-DMA.

[0220] In some embodiments, the cationic lipid and the neutral lipid are present in a molar ratio of 1:1 to 10:1.

[0221] In some embodiments, the cationic lipid and the structural lipid are present in a molar ratio of 1:1 to 5:1.

[0222] In some embodiments, the cationic lipid, the neutral lipid, the structural lipid, and the polymer-conjugated lipid are present in a molar ratio of (25–65):(5–25):(25–70):(0.5–5).

[0223] In some embodiments, the cationic lipid, the neutral lipid, the structural lipid, and the polymer-conjugated lipid are present in a molar ratio of (25–65):(5–25):(25–45):(0.5–5).

[0224] In some embodiments, the cationic lipid, the neutral lipid, the structural lipid, and the polymer-conjugated lipid are present in a molar ratio of 50:10:38.5:1.5 or 49:10:39.5:1.5.

[0225] In some embodiments, the neutral lipid comprises one or more selected from phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, ceramide, sterols, and derivatives thereof.

[0226] In some embodiments, the neutral lipid is selected from one or more of the following: 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-3-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME

16.0PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), dipalmitoylphosphatidylglycerol (DPPG), palmitoyl-oleoylphosphatidylethanolamine (POPE), distearoylphosphatidylethanolamine (DSPE), dipalmitoylphosphatidylethanolamine (DPPE), dimyristoylphosphatidylethanolamine (DMPE), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (SOPE), 1-stearoyl-2-oleoyl-phosphatidylcholine (SOPC), sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyl-oleoylphosphatidylcholine, lysophosphatidylcholine, lysophosphatidylethanolamine (LPE), and mixtures thereof.

[0227] In some embodiments, the neutral lipid is 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and/or 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC).

[0228] In some embodiments, the structural lipid is selected from one or more of the following: cholesterol, non-sterols, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, α -tocopherol, and corticosteroids.

[0229] In some embodiments, the structural lipid is cholesterol.

[0230] In some embodiments, the polymer-conjugated lipid is selected from one or more of the following: PEG-modified phosphatidylethanolamine, PEG-modified phosphatidic acid, PEG-modified ceramide, PEG-modified dialkylamine, PEG-modified diacylglycerol, and PEG-modified dialkylglycerol.

[0231] In some embodiments, the polymer-conjugated lipid is selected from one or more of the following: distearoylphosphatidylethanolamine-polyethylene glycol 2000 (DSPE-PEG2000), dimyristoylglycerol-methoxypolyethylene glycol 2000 (DMG-PEG2000), and methoxypolyethylene glycol bis(tetradecyl)acetamide (ALC-0159).

[0232] The pharmaceutical compositions of the present disclosure include, but are not limited to, solutions, emulsions, and lipid-containing formulations. These compositions can be derived from a variety of components, including but not limited to pre-formed liquids, self-emulsifying solids, and self-emulsifying semi-solids. Particularly preferred are formulations

that target the liver when treating liver diseases (e.g., liver cancer).

[0233] The pharmaceutical compositions of the present disclosure (which may conveniently be provided in unit dosage forms) can be prepared according to conventional techniques well-known in the pharmaceutical industry. Such techniques include a step of combining the active ingredient(s) with the pharmaceutical carrier(s) or excipient(s). In general, these pharmaceutical compositions are prepared by uniformly and intimately combining the active ingredient(s) with a liquid carrier, a finely divided solid carrier, or both, and, if necessary, subsequently shaping the product.

[0234] The pharmaceutical compositions of the present disclosure may be formulated into any of a number of possible dosage forms, including but not limited to tablets, capsules, gel capsules, liquid syrups, soft capsules, suppositories, and enemas. The pharmaceutical compositions of the present disclosure may also be formulated as suspensions in aqueous, non-aqueous, or mixed media. An aqueous suspension may further comprise a substance that increases the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, and/or dextran. The suspension may further comprise a stabilizer.

[0235] Certain pharmaceutical compositions of the present disclosure further incorporate a carrier compound into the formulation. As used herein, the term “carrier compound” or “carrier” may refer to a nucleic acid or analog thereof that is inert (i.e., lacks biological activity on its own) but is recognized as a nucleic acid in biological processes, such as by degrading biologically active nucleic acids or promoting their removal from circulation, thereby reducing the bioavailability of the biologically active nucleic acid. Co-administration of a nucleic acid and a carrier compound (typically with the latter in excess) can result in a significant reduction in the amount of nucleic acid recovered in the liver, kidneys, or other extravascular reservoirs, presumably due to competition for shared receptors between the carrier compound and the nucleic acid. For example, the recovery of partially phosphorothioated dsRNA in liver tissue can be reduced when co-administered with polyinosinic acid, dextran sulfate, polycytidylic acid, or 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid.

[0236] In contrast to carrier compounds, the term “pharmaceutical carrier” or “excipient” refers to a pharmaceutically acceptable solvent, suspending agent, or any other pharmaceutically inert vehicle used to deliver one or more nucleic acids to an animal. The

excipient may be liquid or solid and is selected based on the intended route of administration to provide the desired volume, consistency, and other properties when combined with the nucleic acid and other components of the specific pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to binders (e.g., pregelatinized corn starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates, or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc, silica, colloidal silica, stearic acid, metal stearates, hydrogenated vegetable oils, corn starch, polyethylene glycol, sodium benzoate, or sodium acetate); disintegrants (e.g., starch, sodium starch glycolate); and wetting agents (e.g., sodium lauryl sulfate).

[0237] Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration and do not exhibit toxic interactions with nucleic acids may also be used to formulate the pharmaceutical compositions of the present disclosure. Suitable pharmaceutically acceptable carriers include, but are not limited to water, salt solutions, alcohols, polyethylene glycol, gelatin, lactose, amylose, magnesium stearate, talc, silica, viscous paraffin, hydroxymethyl cellulose, polyvinylpyrrolidone, and the like.

[0238] Formulations for topical administration of nucleic acids may include sterile/non-sterile aqueous/non-aqueous solutions in common solvents such as alcohol, or nucleic acid solutions in liquid or solid oil bases. These solutions may also include buffers, diluents, and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration and do not exhibit toxic interactions with nucleic acids may be used.

[0239] Suitable pharmaceutically acceptable excipients include, but are not limited to water, salt solutions, alcohols, polyethylene glycol, gelatin, lactose, amylose, magnesium stearate, talc, silica, viscous paraffin, hydroxymethyl cellulose, polyvinylpyrrolidone, and the like.

[0240] The dosage forms, carrier compounds, pharmaceutical carriers, excipients, and the like described above are detailed in U.S. Patent No. US10125369B2, which is incorporated herein by reference.

[0241] In another aspect, the present disclosure provides a kit, wherein the kit comprises a kit A, and the kit A comprises one or more of the aforementioned double-stranded RNAi agent,

the conjugate, or the pharmaceutical composition.

[0242] In some embodiments, the kit further comprises a kit B, and the kit B comprises one or both of the following:

[0243] (1) an additional drug that reduces HBV gene expression or a composition comprising the drug that reduces HBV gene expression;

[0244] (2) one or more agents selected from the group consisting of hormone preparations, targeted small molecule agents, proteasome inhibitors, imaging agents, diagnostic agents, chemotherapeutic agents, oncolytic drugs, cytotoxic agents, cytokines, activators of co-stimulatory molecules, inhibitors of inhibitory molecules, and vaccines.

[0245] As used herein, the phrase “an additional drug that reduces HBV gene expression or a composition comprising the drug that reduces HBV gene expression” refers to drugs that do not contain the double-stranded RNAi agent, conjugate, or pharmaceutical composition provided in the present disclosure.

[0246] In another aspect, the present disclosure provides a use of the double-stranded RNAi agent, the conjugate, or the pharmaceutical composition in the preparation of a medicament for preventing and/or treating a disease associated with HBV gene expression.

[0247] In some embodiments, the disease associated with HBV gene expression is selected from the following disease types: chronic hepatitis B, liver fibrosis, liver cirrhosis, liver cancer, acute hepatitis B, and HBV/hepatitis D virus co-infection-associated diseases.

[0248] In another aspect, the present disclosure provides a method for reducing HBV gene expression or inhibiting HBV replication for non-preventive and/or non-therapeutic purposes, wherein the method comprises administering to a sample one or more of the aforementioned double-stranded RNAi agent, conjugate, pharmaceutical composition, or kit.

[0249] As used herein, the term “non-preventive and/or non-therapeutic purpose” refers to cases of reducing HBV gene expression or inhibiting HBV replication, for example, for research purposes in a laboratory setting.

[0250] Nucleotide abbreviations used herein are as follows:

[0251] A = Adenosine-3'-phosphate

[0252] Am = 2'-Methoxyadenosine-3'-phosphate

[0253] Ams = 2'-Methoxyadenosine-3'-phosphorothioate

- [0254] Af = 2'-Fluoroadenosine-3'-phosphate
- [0255] Afs = 2'-Fluoroadenosine-3'-phosphorothioate
- [0256] G = Guanosine-3'-phosphate
- [0257] Gm = 2'-Methoxyguanosine-3'-phosphate
- [0258] Gms = 2'-Methoxyguanosine-3'-phosphorothioate
- [0259] Gf = 2'-Fluoroguanosine-3'-phosphate
- [0260] Gfs = 2'-Fluoroguanosine-3'-phosphorothioate
- [0261] C = Cytidine-3'-phosphate
- [0262] Cm = 2'-Methoxycytidine-3'-phosphate
- [0263] Cms = 2'-Methoxycytidine-3'-phosphorothioate
- [0264] Cf = 2'-Fluorocytidine-3'-phosphate
- [0265] Cfs = 2'-Fluorocytidine-3'-phosphorothioate
- [0266] U = Uridine-3'-phosphate
- [0267] Um = 2'-Methoxyuridine-3'-phosphate
- [0268] Ums = 2'-Methoxyuridine-3'-phosphorothioate
- [0269] Uf = 2'-Fluorouridine-3'-phosphate
- [0270] Ufs = 2'-Fluorouridine-3'-phosphorothioate
- [0271] AmsEVP = 5'-(E)-vinylphosphonate-2'-O-methyl-adenosine-3'-phosphorothioate
- [0272] UmsEVP = 5'-(E)-vinylphosphonate-2'-O-methyl-uridine-3'-phosphorothioate
- [0273] A(gna) = Adenosine-glycol nucleic acid
- [0274] C(gna) = Cytidine-glycol nucleic acid
- [0275] G(gna) = Guanosine-glycol nucleic acid
- [0276] T(gna) = Thymidine-glycol nucleic acid
- [0277] U(gna) = Uridine-glycol nucleic acid
- [0278] dA = Deoxyadenosine-3'-phosphate
- [0279] dG = Deoxyguanosine-3'-phosphate
- [0280] dC = Deoxycytidine-3'-phosphate
- [0281] dT = Deoxythymidine-3'-phosphate
- [0282] The following examples are provided to illustrate the present disclosure but are not intended to limit its scope. Unless otherwise specified, the technical methods used in the

examples are conventional methods well-known to those skilled in the art, and all raw materials are commercially available products.

[0283] Example 1: Synthesis of small interfering oligonucleotides modified using the DV29P template

[0284] A total of 36 siRNA unmodified sequences were designed, with Alnylam's VIR-2218 used as the positive control (referred to as APC-VIR in this example) and ANC-DV29P used as the negative control. The unmodified sequences were modified using the DV29P template. In the sense strand, positions 7, 9, 11, and 17 were modified with 2'-F, while the remaining positions were modified with 2'-OMe. In the antisense strand, positions 2, 4, 5, 6, 14, and 16 were modified with 2'-F, while the remaining positions were modified with 2'-OMe. Additionally, the 5' end of the sense strand has two phosphorothioate modifications. The antisense strand has two phosphorothioate modifications at both the 5' and 3' ends, as well as one EVP modification at the 5' end. The sequences of the DV29P template-modified siRNAs are listed in Table 1.

[0285] 1. Synthesis of the DV29P template-modified sequence A194-DV29P

[0286] In table 1, the unmodified sequence of the small interfering ribonucleic acid (siRNA) with the sequence code A194-DV29P is as follows:

[0287] Sense strand: 5'- UCGUGUUACAGGCGGGGUUUU -3' (SEQ ID NO: 15)

[0288] Antisense strand: 5'- AAAACCCCGCCUGUAACACGAGA -3' (SEQ ID NO: 47)

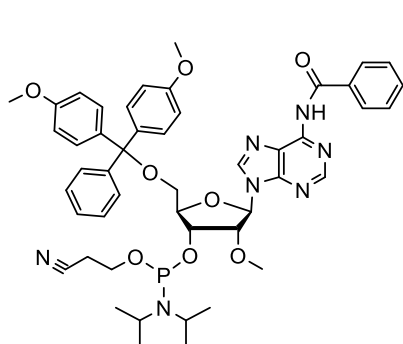
[0289] In the sense strand, positions 7, 9, 11, and 17 were modified with 2'-F, while the remaining positions were modified with 2'-OMe. In the antisense strand, positions 2, 4, 5, 6, 14, and 16 were modified with 2'-F, while the remaining positions were modified with 2'-OMe. Additionally, the 5' end of the sense strand has two phosphorothioate modifications. The antisense strand has two phosphorothioate modifications at both the 5' and 3' ends, as well as one EVP modification at the 5' end.

[0290] Instruments and reagents: The DNA/RNA automatic synthesizer used is the Tsingke 192 P model. The solid-phase support is a universal support composed of cross-linked polystyrene beads, specifically the Primer Support 5G Unylinker 350 (manufactured by Cytiva).

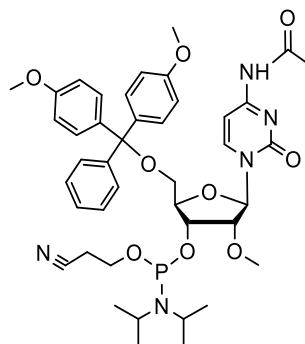
[0291] Preparation method:

[0292] The following nucleotide monomer solutions were prepared using acetonitrile as the

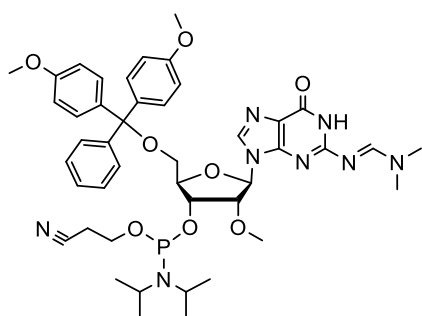
solvent, with a monomer concentration of 0.15 M: DMT-A-OMe phosphoramidite monomer (formula 1), DMT-C-OMe phosphoramidite monomer (formula 2), DMT-G-OMe phosphoramidite monomer (formula 3), DMT-U-OMe phosphoramidite monomer (formula 4), DMT-A-F phosphoramidite monomer (formula 5), DMT-C-F phosphoramidite monomer (formula 6), DMT-G-F phosphoramidite monomer (formula 7), DMT-U-F phosphoramidite monomer (formula 8), (E)-vinylphosphonate-A-OMe phosphoramidite monomer (formula 9), (E)-vinylphosphonate-U-OMe phosphoramidite monomer (formula 10).



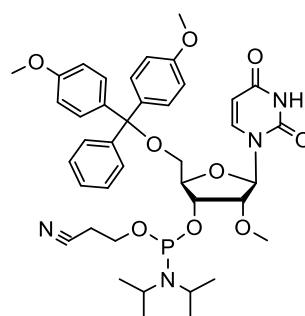
formula 1



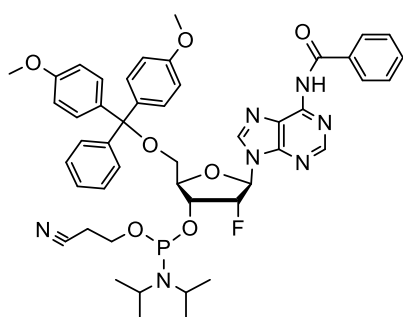
formula 2



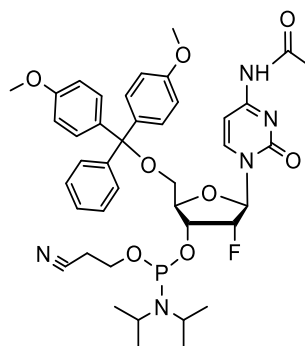
formula 3



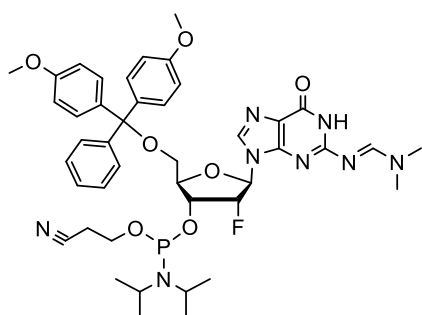
formula 4



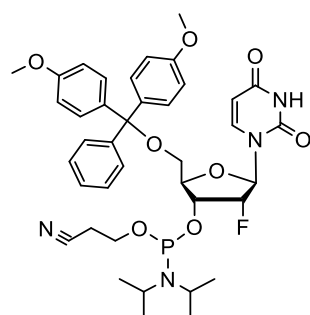
formula 5



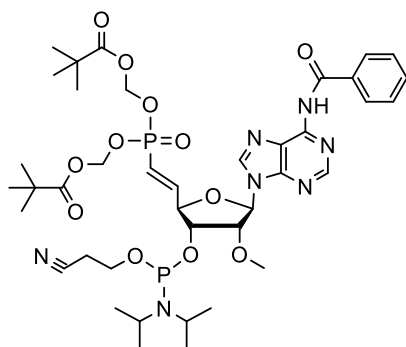
formula 6



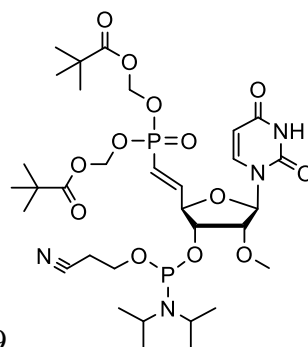
formula 7



formula 8

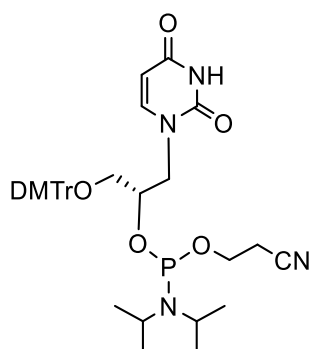


formula 9

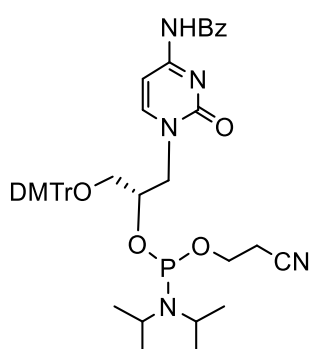


formula 10

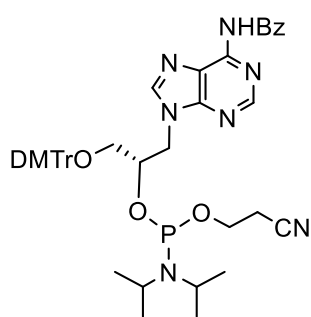
[0293] The 7th base from the 5' end of the antisense strand in the positive control sequence APC-VIR is modified with glycol nucleic acid (GNA), and the GNA monomer structures are as follows:



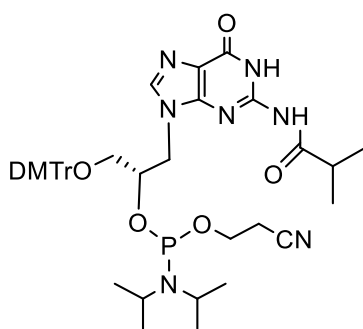
(S)-GNA-U



(S)-GNA-C



(S)-GNA-A



(S)-GNA-G

[0294] Preparation steps are as follows:

[0295] (1) Deprotection:

[0296] A 3% dichloroacetic acid solution in toluene was used as the deprotection reagent to

remove the DMT protecting group. Subsequently the product was washed with acetonitrile.

[0297] (2) Coupling:

[0298] A 0.25 M solution of 5-ethylthio-1H-tetrazole in acetonitrile was used as the activator for coupling each nucleotide monomer solution in acetonitrile. Subsequently the product was rinsed with acetonitrile.

[0299] (3) Oxidation/sulfurization:

[0300] Oxidation: A 0.05 M iodine solution in pyridine/water (90/10, v/v) was used as the oxidizing agent for oxidation. Subsequently the product was rinsed with acetonitrile.

[0301] Sulfurization: A 3% solution of hydrogen xanthate in pyridine was used as the sulfurizing agent for sulfurization. Subsequently the product was rinsed with acetonitrile.

[0302] (4) Hydroxyl protection:

[0303] A 10% acetic anhydride solution in tetrahydrofuran (CAP A) and a mixture of tetrahydrofuran/pyridine/N-methylimidazole (74/10/16, v/v/v) (CAP B) were used as hydroxyl protection reagents for hydroxyl protection. Subsequently the product was rinsed with acetonitrile.

[0304] The above steps were repeated in cycles according to the designed sequence to obtain the fully protected product.

[0305] (5) A 3% dichloroacetic acid solution in toluene was used as the deprotection reagent to remove the DMT protecting group from the last nucleotide. Subsequently the product was washed with acetonitrile.

[0306] (6) Ammonolysis and purification:

[0307] The solid-phase support was transferred to a reactor, and concentrated aqueous ammonia (25-28%) was added. The mixture was maintained at 60°C for 12 hours for ammonolysis. After cooling to room temperature, the mixture was filtered and was washed with a mixture of purified water and ethanol. The combined filtrate was passed through a chromatography column, concentrated, and lyophilized to obtain the product.

[0308] (7) Annealing

The purified sense strand and antisense strand were mixed in a 1:1 ratio, heated to 95°C for 3 minutes, and then slowly cooled to room temperature to form the double-stranded product.

[0309] A194-DV29P purity: 90.52%; measured molecular weight: 14,727.09.

[0310] 2. Synthesis of other sequences

[0311] The synthesis of other sequences in Table 1 was carried out according to the method described above.

[0312] Table 1: DV29P template-modified siRNA sequences

unmodified sequence code	Sequence code	Sense strand sequence 5'-3'	Seque nce ID No	Correspon ding unmodifie d sequence ID No	Antisense strand sequence 5'-3'	Seque nce ID No	Correspon ding basic sequence ID No
B207S	B207S- DV29P	Gms-Gms-Gm-Gm- Um-Uf-Um-Uf-Um- Cf-Um-Um-Gm- Um-Um-Gf-Am- Cm-Am-Am	81	1	UmsEVP-Ufs-Gm-Uf- Cf-Af-Am-Cm-Am- Am-Gm-Am-Am-Af- Am-Af-Cm-Cm-Cm- Cms-Gms-Cm	179	8
A1550	A1550- DV29P	Cms-Cms-Cm-Gm- Um-Cm-Uf-Gm-Uf- Gm-Cf-Cm-Um- Um-Cm-Um-Cf- Am-Um-Cm-Um	86	2	AmsEVP-Gfs-Am-Uf- Gf-Af-Gm-Am-Am- Gm-Gm-Cm-Am-Cf- Am-Gf-Am-Cm-Gm- Gm-Gms-Gms-Am	187	9
B1572	B1572- DV29P	Cms-Cms-Gm-Gm- Am-Cm-Cf-Gm-Uf- Gm-Uf-Gm-Cm- Am-Cm-Um-Uf- Cm-Gm-Cm-Um	91	3	AmsEVP-Gfs-Cm-Gf- Af-Af-Gm-Um-Gm- Cm-Am-Cm-Am-Cf- Gm-Gf-Um-Cm-Cm- Gm-Gms-Cms-Am	195	10
B1575	B1575- DV29P	Gms-Ams-Cm-Cm- Gm-Um-Gf-Um-Gf- Cm-Af-Cm-Um- Um-Cm-Gm-Cf- Um-Um-Cm-Am	96	4	UmsEVP-Gfs-Am-Af- Gf-Cf-Gm-Am-Am- Gm-Um-Gm-Cm-Af- Cm-Af-Cm-Gm-Gm- Um-Cms-Cms-Gm	201	11
A1573	A1573- DV29P	Cms-Gms-Gm-Am- Cm-Cm-Gf-Um-Gf- Um-Gf-Cm-Am- Cm-Um-Um-Cf- Gm-Cm-Um-Um	101	5	AmsEVP-Afs-Gm-Cf- Gf-Af-Am-Gm-Um- Gm-Cm-Am-Cm-Af- Cm-Gf-Gm-Um-Cm- Cm-Gms-Gms-Cm	207	12
B418S	B418S- DV29P	Cms-Ums-Gm-Cm- Um-Af-Um-Gf-Cm- Cf-Um-Cm-Am- Um-Cm-Uf-Um- Cm-Um-Um	106	6	AmsEVP-Afs-Gm-Af- Af-Gf-Am-Um-Gm- Am-Gm-Gm-Cm-Af- Um-Af-Gm-Cm-Am- Gms-Cms-Am	213	13
A416	A416- DV29P	Ums-Gms-Cm-Um- Gm-Cm-Uf-Am-Uf-	111	7	AmsEVP-Gfs-Am-Af- Gf-Af-Um-Gm-Am-	219	14

		Gm-Cf-Cm-Um- Cm-Am-Um-Cf- Um-Um-Cm-Um			Gm-Gm-Cm-Am-Uf- Am-Gf-Cm-Am-Gm- Cm-Ams-Gms-Gm		
A194	A194- DV29P	Ums-Cms-Gm-Um- Gm-Um-Uf-Am-Cf- Am-Gf-Gm-Cm- Gm-Gm-Gm-Gf- Um-Um-Um-Um	156	15	AmsEVP-Afs-Am-Af- Cf-Cf-Cm-Cm-Gm- Cm-Cm-Um-Gm-Uf- Am-Af-Cm-Am-Cm- Gm-Ams-Gms-Am	273	47
A200	A200- DV29P	Ums-Ams-Cm-Am- Gm-Gm-Cf-Gm-Gf- Gm-Gf-Um-Um- Um-Um-Um-Cf- Um-Um-Gm-Um	157	16	AmsEVP-Cfs-Am-Af- Gf-Af-Am-Am-Am- Am-Cm-Cm-Cm-Cf- Gm-Cf-Cm-Um-Gm- Um-Ams-Ams-Cm	274	48
A203	A203- DV29P	Ams-Gms-Gm-Cm- Gm-Gm-Gf-Gm-Uf- Um-Uf-Um-Um- Cm-Um-Um-Gf- Um-Um-Gm-Am	158	17	UmsEVP-Cfs-Am-Af- Cf-Af-Am-Gm-Am- Am-Am-Am-Am-Cf- Cm-Cf-Cm-Gm-Cm- Cm-Ums-Gms-Um	275	49
A205	A205- DV29P	Gms-Cms-Gm-Gm- Gm-Gm-Uf-Um-Uf- Um-Uf-Cm-Um- Um-Gm-Um-Uf- Gm-Am-Cm-Am	116	18	UmsEVP-Gfs-Um-Cf- Af-Af-Cm-Am-Am- Gm-Am-Am-Am-Af- Am-Cf-Cm-Cm-Cm- Gm-Cms-Cms-Um	225	50
A206	A206- DV29P	Cms-Gms-Gm-Gm- Gm-Um-Uf-Um-Uf- Um-Cf-Um-Um- Gm-Um-Um-Gf- Am-Cm-Am-Am	159	19	UmsEVP-Ufs-Gm-Uf- Cf-Af-Am-Cm-Am- Am-Gm-Am-Am-Af- Am-Af-Cm-Cm-Cm- Cm-Gms-Cms-Cm	276	51
A248	A248- DV29P	Gms-Ums-Cm-Um- Am-Gm-Af-Cm-Uf- Cm-Gf-Um-Gm- Gm-Um-Gm-Gf- Am-Cm-Um-Um	160	20	AmsEVP-Afs-Gm-Uf- Cf-Cf-Am-Cm-Cm- Am-Cm-Gm-Am-Gf- Um-Cf-Um-Am-Gm- Am-Cms-Ums-Cm	277	52
A255	A255- DV29P	Cms-Ums-Cm-Gm- Um-Gm-Gf-Um-Gf- Gm-Af-Cm-Um- Um-Cm-Um-Cf- Um-Cm-Am-Am	161	21	UmsEVP-Ufs-Gm-Af- Gf-Af-Gm-Am-Am- Gm-Um-Cm-Cm-Af- Cm-Cf-Am-Cm-Gm- Am-Gms-Ums-Cm	278	53
A256	A256- DV29P	Ums-Cms-Gm-Um- Gm-Gm-Uf-Gm-Gf- Am-Cf-Um-Um- Cm-Um-Cm-Uf- Cm-Am-Am-Um	162	22	AmsEVP-Ufs-Um-Gf- Af-Gf-Am-Gm-Am- Am-Gm-Um-Cm-Cf- Am-Cf-Cm-Am-Cm- Gm-Ams-Gms-Um	279	54

A258	A258-DV29P	Gms-Ums-Gm-Gm-Um-Gm-Gf-Am-Cf-Um-Uf-Cm-Um-Cm-Um-Am-Um-Um-Um	163	23	AmsEVP-Afs-Am-Uf-Uf-Gf-Am-Gm-Am-Gm-Am-Am-Gm-Uf-Cm-Cf-Am-Cm-Cm-Am-Cms-Gms-Am	280	55
A259	A259-DV29P	Ums-Gms-Gm-Um-Gm-Gm-Af-Cm-Uf-Um-Cf-Um-Cm-Um-Cm-Am-Af-Um-Um-Um-Um	164	24	AmsEVP-Afs-Am-Af-Uf-Uf-Gm-Am-Gm-Am-Gm-Am-Am-Gf-Um-Cf-Cm-Am-Cm-Cm-Ams-Cms-Gm	281	56
A261	A261-DV29P	Gms-Ums-Gm-Gm-Am-Cm-Uf-Um-Cf-Um-Cf-Um-Cm-Am-Am-Um-Uf-Um-Um-Cm-Um	126	25	AmsEVP-Gfs-Am-Af-Af-Af-Um-Um-Gm-Am-Gm-Am-Gm-Af-Am-Gf-Um-Cm-Cm-Am-Cms-Cms-Am	237	57
A381	A381-DV29P	Gms-Ums-Gm-Um-Cm-Um-Gf-Cm-Gf-Gm-Cf-Gm-Um-Um-Um-Um-Af-Um-Cm-Am-Um	165	26	AmsEVP-Ufs-Gm-Af-Uf-Af-Am-Am-Am-Cm-Gm-Cm-Cm-Gf-Cm-Af-Gm-Am-Cm-Am-Cms-Ams-Um	282	58
A413	A413-DV29P	Ums-Cms-Cm-Um-Gm-Cm-Uf-Gm-Cf-Um-Af-Um-Gm-Cm-Cm-Um-Cf-Am-Um-Cm-Um	166	27	AmsEVP-Gfs-Am-Uf-Gf-Af-Gm-Gm-Cm-Am-Um-Am-Gm-Cf-Am-Gf-Cm-Am-Gm-Gm-Ams-Ums-Gm	283	59
A463	A463-DV29P	Ams-Ums-Gm-Um-Um-Gm-Cf-Cm-Cf-Gm-Uf-Um-Um-Gm-Um-Cm-Cf-Um-Cm-Um-Am	167	28	UmsEVP-Afs-Gm-Af-Gf-Gf-Am-Cm-Am-Am-Am-Cm-Gm-Gf-Gm-Cf-Am-Am-Cm-Am-Ums-Ams-Cm	284	60
A536	A536-DV29P	Cms-Ums-Gm-Cm-Um-Cm-Af-Am-Gf-Gm-Af-Am-Cm-Cm-Um-Cm-Uf-Am-Um-Gm-Um	168	29	AmsEVP-Cfs-Am-Uf-Af-Gf-Am-Gm-Gm-Um-Um-Cm-Cm-Uf-Um-Gf-Am-Gm-Cm-Am-Gms-Ums-Am	285	61
A707	A707-DV29P	Ums-Ams-Gm-Gm-Gm-Cm-Uf-Um-Uf-Cm-Cf-Cm-Cm-Cm-Am-Cm-Uf-Gm-Um-Um-Um	169	30	AmsEVP-Afs-Am-Cf-Af-Gf-Um-Gm-Gm-Gm-Gm-Gm-Am-Af-Am-Gf-Cm-Cm-Cm-Um-Ams-Cms-Gm	286	62
A734	A734-DV29P	Cms-Ams-Gm-Um-Um-Am-Uf-Am-Uf-Gm-Gf-Am-Um	170	31	AmsEVP-Cfs-Cm-Af-Cf-Af-Um-Cm-Am-Um-Cm-Cm-Am-Uf	287	63

		Gm-Am-Um-Gf- Um-Gm-Gm-Um			Am-Uf-Am-Am-Cm- Um-Gms-Ams-Am		
A1260	A1260- DV29P	Cms-Ums-Gm-Cm- Cm-Gm-Af-Um-Cf- Cm-Af-Um-Am- Cm-Um-Gm-Cf- Gm-Gm-Am-Am	171	32	UmsEVP-Ufs-Cm-Cf- Gf-Cf-Am-Gm-Um- Am-Um-Gm-Gm-Af- Um-Cf-Gm-Gm-Cm- Am-Gms-Ams-Gm	288	64
A1266	A1266- DV29P	Ams-Ums-Cm-Cm- Am-Um-Af-Cm-Uf- Gm-Cf-Gm-Gm- Am-Am-Cm-Uf- Cm-Cm-Um-Am	172	33	UmsEVP-Afs-Gm-Gf- Af-Gf-Um-Um-Cm- Cm-Gm-Cm-Am-Gf- Um-Af-Um-Gm-Gm- Am-Ums-Cms-Gm	289	65
A1518	A1518- DV29P	Ams-Cms-Cm-Am- Cm-Gm-Gf-Gm-Gf- Cm-Gf-Cm-Am- Cm-Cm-Um-Cf- Um-Cm-Um-Um	173	34	AmsEVP-Afs-Gm-Af- Gf-Af-Gm-Gm-Um- Gm-Cm-Gm-Cm-Cf- Cm-Cf-Gm-Um-Gm- Gm-Ums-Cms-Gm	290	66
A1520	A1520- DV29P	Cms-Ams-Cm-Gm- Gm-Gm-Gf-Cm-Gf- Cm-Af-Cm-Cm- Um-Cm-Um-Cf- Um-Um-Um-Am	174	35	UmsEVP-Afs-Am-Af- Gf-Af-Gm-Am-Gm- Gm-Um-Gm-Cm-Gf- Cm-Cf-Cm-Cm-Gm- Um-Gms-Gms-Um	291	67
A1547	A1547- DV29P	Cms-Ums-Cm-Cm- Cm-Cm-Gf-Um-Cf- Um-Gf-Um-Gm- Cm-Cm-Um-Uf- Cm-Um-Cm-Am	175	36	UmsEVP-Gfs-Am-Gf- Af-Af-Gm-Gm-Cm- Am-Cm-Am-Gm-Af- Cm-Gf-Gm-Gm-Gm- Am-Gms-Ums-Cm	292	68
A1548	A1548- DV29P	Ums-Cms-Cm-Cm- Cm-Gm-Uf-Cm-Uf- Gm-Uf-Gm-Cm- Cm-Um-Um-Cf- Um-Cm-Am-Um	176	37	AmsEVP-Ufs-Gm-Af- Gf-Af-Am-Gm-Gm- Cm-Am-Cm-Am-Gf- Am-Cf-Gm-Gm-Gm- Gm-Ams-Gms-Um	293	69
B208	B208- DV29P	Gms-Gms-Gm-Um- Um-Um-Uf-Um-Cf- Um-Uf-Gm-Um- Um-Gm-Am-Cf- Am-Am-Gm-Am	121	38	UmsEVP-Cfs-Um-Uf- Gf-Uf-Cm-Am-Am- Cm-Am-Am-Gm-Af- Am-Af-Am-Am-Cm- Cm-Cms-Cms-Gm	231	70
B262	B262- DV29P	Ums-Gms-Gm-Am- Cm-Um-Uf-Cm-Uf- Cm-Uf-Cm-Am- Am-Um-Um-Uf- Um-Cm-Um-Am	131	39	UmsEVP-Afs-Gm-Af- Af-Af-Am-Um-Um- Gm-Am-Gm-Am-Gf- Am-Af-Gm-Um-Cm- Cm-Ams-Cms-Gm	243	71
B264	B264- DV29P	Gms-Ams-Cm-Um- Um-Cm-Uf-Cm-Uf-	136	40	UmsEVP-Cfs-Um-Af- Gf-Af-Am-Am-Am-	249	72

		Cm-Af-Am-Um- Um-Um-Um-Cf- Um-Am-Gm-Am			Um-Um-Gm-Am-Gf- Am-Gf-Am-Am-Gm- Um-Cms-Cms-Am		
B1556	B1556- DV29P	Ums-Gms-Um-Gm- Cm-Cm-Uf-Um-Cf- Um-Cf-Am-Um- Cm-Um-Gm-Cf- Cm-Gm-Gm-Am	141	41	UmsEVP-Cfs-Cm-Gf- Gf-Cf-Am-Gm-Am- Um-Gm-Am-Gm-Af- Am-Gf-Gm-Cm-Am- Cm-Ams-Gms-Am	255	73
B1560	B1560- DV29P	Cms-Cms-Um-Um- Cm-Um-Cf-Am-Uf- Cm-Uf-Gm-Cm- Cm-Gm-Gm-Af- Cm-Cm-Gm-Um	146	42	AmsEVP-Cfs-Gm-Gf- Uf-Cf-Cm-Gm-Gm- Cm-Am-Gm-Am-Uf- Gm-Af-Gm-Am-Am- Gm-Gms-Cms-Am	261	74
B416S	B416S- DV29P	Ums-Gms-Cm-Um- Gm-Cf-Um-Af-Um- Gf-Cm-Cm-Um- Cm-Am-Uf-Cm- Um-Um-Am	151	43	UmsEVP-Afs-Am-Gf- Af-Uf-Gm-Am-Gm- Gm-Cm-Am-Um-Af- Gm-Cf-Am-Gm-Cm- Ams-Gms-Gm	267	75
APC	APC-VIR	Gms-Ums-Gm-Um- Gf-Cm-Af-Cf-Uf- Um-Cm-Gm-Cm- Um-Um-Cm-Am- Cm-Am	177	45	Ums-Gfs-Um-Gm-Am- A(gna)-Gm-Cf-Gf- Am-Am-Gm-Um-Gf- Cm-Af-Cm-Am-Cms- Ums-Um	294	79
ANC	ANC- DV29P	Gms-Gms-Um-Cm- Um-Cm-Uf-Um-Cf- Um-Af-Um-Cm- Gm-Am-Um-Cf- Um-Um-Am-Um	178	46	AmsEVP-Ufs-Am-Af- Gf-Af-Um-Cm-Gm- Am-Um-Am-Gm-Af- Am-Gf-Am-Gm-Am- Cm-Cms-Cms-Am	295	80

[0313] Example 2: Inhibitory effects of DV29P template-modified sequences on HBsAg and HBeAg

[0314] The DV29P template-modified siRNA sequences synthesized in Example 1 were transfected into HepG2.2.15 cells using lipid nanoparticles (LNPs). The inhibitory effects of the sequences on HBsAg and HBeAg were then evaluated using ELISA.

[0315] 1. Experimental materials

[0316] Test samples: DV29P template-modified siRNA sequences listed in Table 1 (synthesized in Example 1).

[0317] Cell line: HepG2.2.15 cells.

[0318] Vehicle reagents: Sterile nuclease-free water and Gibco™ Opti-MEM™ (Thermo

Fisher Scientific).

[0319] 2. Experimental methods

[0320] The inhibitory effects of the test samples on HBsAg and HBeAg in HepG2.2.15 cells were assessed using ELISA.

[0321] 2.1 Cell culture

[0322] Subculture: HepG2.2.15 cells were subcultured in DMEM/F12 medium containing 10% fetal bovine serum, 370 µg/mL Geneticin, 1% L-glutamine, 1% non-essential amino acids, and 1% penicillin-streptomycin. The cells were maintained in a cell incubator at 37°C with 5% CO₂ and subcultured every three days. For subculturing, the cells were detached using 0.25% trypsin, centrifuged at 800 rpm for 3 minutes, and the supernatant was discarded. Fresh medium was then added for further subculture.

[0323] Plating: HepG2.2.15 cells were plated in DMEM/F12 medium containing 10% fetal bovine serum (FBS), 1% L-glutamine, 1% non-essential amino acids, and 1% penicillin-streptomycin.

[0324] 2.2 Cell transfection

[0325] Preparation of transfection mixture: Lipofectamine™ RNAiMAX (Thermo Fisher Scientific) and Opti-MEM were mixed in a 2:98 ratio and vortexed thoroughly.

[0326] Preparation of transfection complex: 30 µL of siRNA solution diluted in Opti-MEM was combined with 30 µL of the transfection mixture at a 1:1 (v/v) ratio, vortexed, and incubated at room temperature for 15 minutes to form the transfection complex.

[0327] Preparation of control transfection reagent: 15 µL of the prepared transfection mixture was added to 15 µL of Opti-MEM. The mixture was vortexed and then incubated at room temperature for 15 minutes.

[0328] The prepared transfection complex was added to a 96-well cell culture plate (15 µL per well, with three replicates per sequence), resulting in a final siRNA concentration of 0.3 nM per well. 135 µL of cell suspension (containing 2.25×10^4 cells) was added to each well. After mixing using the cross-mixing method, the plate was placed in a 37°C, 5% CO₂ cell incubator for incubation.

[0329] 2.3 Detection of HBsAg and HBeAg in cell supernatant

[0330] 1) Collection of cell supernatant

[0331] a. On day 3 post-transfection, the culture medium was replaced by removing the cell supernatant and adding 150 µL/well of fresh medium for continued culture.

[0332] b. On day 6 post-transfection, the cell supernatant was collected for the detection of HBsAg and HBeAg levels.

[0333] 2) Quantitative detection of HBsAg and HBeAg

[0334] The concentrations of HBsAg and HBeAg were measured using the Hepatitis B Virus E Antigen Detection Kit (Autobio CL0310) and the Hepatitis B Virus Surface Antigen Detection Kit (Autobio CL0312). The specific steps are as follows:

[0335] a. The kits and test samples were equilibrated to room temperature.

[0336] b. Test samples, standards, negative controls, and positive controls were added to the plate wells at 50 µL each.

[0337] c. 50 µL of enzyme conjugate was added to each well. After thorough mixing, the plate was incubated at 37°C for 60 minutes.

[0338] d. The liquid in the wells was removed, and the plate was washed 5 times with wash buffer. Finally, the plate was patted dry on absorbent paper.

[0339] e. Luminescent substrates A and B were mixed in equal proportions, and added at 50 µL/ well, followed by a 3-minute incubation at room temperature in the dark.

[0340] f. The luminescence values were measured using a microplate reader.

[0341] 2.4 Data processing

[0342] The inhibition rates of HBsAg and HBeAg were calculated using the following formulas:

[0343] HBsAg inhibition rate (%) = $(1 - \text{HBsAg expression level of sample} / \text{HBsAg expression level of transfection control in the same plate}) \times 100\%$;

[0344] HBeAg inhibition rate (%) = $(1 - \text{HBeAg expression level of sample} / \text{HBeAg expression level of transfection control in the same plate}) \times 100\%$.

[0345] The relative inhibition rate of HBsAg compared to the positive control (with the positive control inhibition rate set as 1) = HBsAg inhibition rate of sample (%) / HBsAg inhibition rate of positive control in the same plate (%). It should be noted that a value greater than 1 indicates that the sequence has a better inhibitory effect on HBsAg than the positive control, while a value less than 1 indicates that the sequence has a lower inhibitory efficiency

on HBsAg compared to the positive control.

[0346] The relative inhibition rate of HBeAg compared to the positive control (with the positive control inhibition rate set as 1) = HBeAg inhibition rate of sample (%) / HBeAg inhibition rate of positive control in the same plate (%). It should be noted that a value greater than 1 indicates that the sequence has a better inhibitory effect on HBeAg than the positive control, while a value less than 1 indicates that the sequence has a lower inhibitory efficiency on HBeAg compared to the positive control.

[0347] 2.5 IC50 experiment

[0348] In this experiment, the concentration of each sequence started at 10 nM, and was diluted 4-fold serially, resulting in 8 concentration points (10 nM, 2.5 nM, 0.625 nM, 0.15625 nM, 39.06 pM, 9.77 pM, 2.44 pM, and 0.61 pM). The inhibition rates of each sequence at these concentrations were measured, plotted, and used to calculate the IC50 values for each sequence and the positive control.

[0349] 2.6 Cytotoxicity assay

[0350] During the IC50 experiment, after collecting the cell supernatant, cell viability was measured using the CellTiter-Glo® (Promega) kit. The method is briefly described as follows: The CellTiter-Glo reagent was mixed with the culture medium at a 1:1 ratio, and 100 µL was added to each well. After incubating at room temperature for 10 minutes, the luminescence signal was measured using a microplate reader.

[0351] The cell viability was calculated using the formula:

[0352] cell viability (%) = (sample signal value – average blank control value) / (average transfection control value – average blank control value) × 100%.

[0353] 3. Experimental results

[0354] The experimental results demonstrated that the sequences B207S-DV29P, B1572-DV29P, B1575-DV29P, B418S-DV29P, A206-DV29P, A416-DV29P, A1548-DV29P, A1550-DV29P, and A1573-DV29P exhibited significantly superior inhibitory effects on HBsAg compared to other sequences, as well as significantly better inhibitory effects on HBeAg compared to other sequences.

[0355] The inhibition rates of each sequence on the expression of HBsAg and HBeAg at a single concentration, as well as their relative inhibition rates compared to the positive control

sequence APC-VIR, are shown in Tables 3, 4, 5, and 6.

[0356] The single-concentration screening results indicated that certain sequences modified with the template DV29P, including B207S-DV29P, B1572-DV29P, B1575-DV29P, B418S-DV29P, A206-DV29P, A416-DV29P, A1548-DV29P, A1550-DV29P, and A1573-DV29P (specific sequences are listed in Table 2), ranked higher in terms of inhibition rates for both HBsAg and HBeAg compared to the positive control APC-VIR.

[0357] The IC₅₀ experimental results and cytotoxicity results for each sequence are as shown in Table 7. The IC₅₀ results demonstrated that the 11 candidate sequences modified with the emplate DV29P designed in the present disclosure exhibited IC₅₀ values for HBsAg ranging from 0.01 to 0.08 nM, all of which were superior to the positive control's IC₅₀ value of 0.1777 nM. For example, the IC₅₀ values for A261-DV29P, A1573-DV29P, and A206-DV29P were 0.0169 nM, 0.0226 nM, and 0.0226 nM, respectively. These sequences effectively inhibited HBsAg expression at low concentrations. For HBeAg, the IC₅₀ values of these 11 candidate sequences ranged from 0.06 to 0.4 nM, all of which were superior to the positive control's IC₅₀ value of 0.7685 nM. For example, the IC₅₀ values for A1573-DV29P, A206-DV29P, and A1550-DV29P were 0.0684 nM, 0.1105 nM, and 0.1202 nM, respectively. The cytotoxicity results showed that among the 11 sequences, except for sequence A416-DV29P, which exhibited a cell viability of 81.82% at the highest tested concentration of 10 nM, all other sequences demonstrated cell viabilities greater than 90%, indicating no significant cytotoxicity. This suggests that the sequences have a wide concentration range for selection.

[0358] The selection criteria prioritized the inhibitory effect on HBsAg while also considering the inhibitory effect on HBeAg. Based on the comprehensive results, 9 sequences were selected from the 11 for further optimization and design. The sequences B207S-DV29P, B1572-DV29P, B1575-DV29P, B418S-DV29P, A206-DV29P, A416-DV29P, A1548-DV29P, A1550-DV29P, and A1573-DV29P were identified as candidate sequences.

[0359] Table 2. siRNA sequences with superior inhibition rates for HBsAg and HBeAg compared to the positive control

Sequence code	Sense strand sequence 5'-3'	Sequence ID No	Unmodified sequence ID No.	Antisense strand sequence 5'-3'	Sequence ID No.	Unmodified sequence ID No.
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B207S-DV29P	Gms-Gms-Gm-Gm-Um-Uf-Um-Uf-Um-Cf-Um-Um-Gm-Um-Um-Gf-Am-Cm-Am-Am	81	1	UmsEVP-Ufs-Gm-Uf-Cf-Af-Am-Cm-Am-Am-Gm-Am-Am-Af-Am-Af-Cm-Cm-Cm-Cms-Gms-Cm	179	8
B1572-DV29P	Cms-Cms-Gm-Gm-Am-Cm-Cf-Gm-Uf-Gm-Uf-Gm-Cm-Am-Cm-Um-Uf-Cm-Gm-Cm-Um	91	3	AmsEVP-Gfs-Cm-Gf-Af-Af-Gm-Um-Gm-Cm-Am-Cm-Am-Cf-Gm-Gf-Um-Cm-Cm-Gm-Gms-Cms-Am	195	10
B1575-DV29P	Gms-Ams-Cm-Cm-Gm-Um-Gf-Um-Gf-Cm-Af-Cm-Um-Um-Cm-Gm-Cf-Um-Um-Cm-Am	96	4	UmsEVP-Gfs-Am-Af-Gf-Cf-Gm-Am-Am-Gm-Um-Gm-Cm-Af-Cm-Af-Cm-Gm-Gm-Um-Cms-Cms-Gm	201	11
B418S-DV29P	Cms-Ums-Gm-Cm-Um-Af-Um-Gf-Cm-Cf-Um-Cm-Am-Um-Cm-Uf-Um-Cm-Um-Um	106	6	AmsEVP-Afs-Gm-Af-Af-Gf-Am-Um-Gm-Am-Gm-Gm-Cm-Af-Um-Af-Gm-Cm-Am-Gms-Cms-Am	213	13
A206-DV29P	Cms-Gms-Gm-Gm-Gm-Um-Uf-Um-Uf-Um-Cf-Um-Um-Gm-Um-Um-Gf-Am-Cm-Am-Am	159	19	UmsEVP-Ufs-Gm-Uf-Cf-Af-Am-Cm-Am-Am-Gm-Am-Am-Af-Am-Af-Cm-Cm-Cm-Cm-Gms-Cms-Cm	276	51
A416-DV29P	Ums-Gms-Cm-Um-Gm-Cm-Uf-Am-Uf-Gm-Cf-Cm-Um-Cm-Am-Um-Cf-Um-Um-Cm-Um	111	7	AmsEVP-Gfs-Am-Af-Gf-Af-Um-Gm-Am-Gm-Gm-Cm-Am-Uf-Am-Gf-Cm-Am-Gm-Cm-Ams-Gms-Gm	219	14
A1548-DV29P	Ums-Cms-Cm-Cm-Cm-Gm-Uf-Cm-Uf-Gm-Uf-Gm-Cm-Cm-Um-Um-Cf-Um-Cm-Am-Um	176	37	AmsEVP-Ufs-Gm-Af-Gf-Af-Am-Gm-Gm-Cm-Am-Cm-Am-Gf-Am-Cf-Gm-Gm-Gm-Gm-Ams-Gms-Um	293	69
A1550-DV29P	Cms-Cms-Cm-Gm-Um-Cm-Uf-Gm-Uf-Gm-Cf-Cm-Um-Um-Cm-Uf-Am-Um-Cm-Um	86	2	AmsEVP-Gfs-Am-Uf-Gf-Af-Gm-Am-Am-Gm-Gm-Cm-Am-Cf-Am-Gf-Am-Cm-Gm-Gm-Gms-Gms-Am	187	9
A1573-DV29P	Cms-Gms-Gm-Am-Cm-Cm-Gf-Um-Gf-Um-Gf-Cm-Am	101	5	AmsEVP-Afs-Gm-Cf-Gf-Af-Am-Gm-Um-Gm-Cm-Am-Cm-Af	207	12

	Cm-Um-Um-Cf- Gm-Cm-Um-Um			Cm-Gf-Gm-Um-Cm- Cm-Gms-Gms-Cm		
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[0360] (i) Inhibition of HBV gene expression by sequences modified with the DV29P template at a single concentration

[0361] (1) Sequences exhibiting superior inhibition of HBsAg compared to the positive control are shown in Table 3, including B207S-DV29P, B1575-DV29P, B1572-DV29P, B418S-DV29P, A261-DV29P, A206-DV29P, A1550-DV29P, A1573-DV29P, A1548-DV29P, and A416-DV29P. Among these, the four sequences B207S-DV29P, B1575-DV29P, B1572-DV29P, and B418S-DV29P demonstrated HBsAg inhibition rates exceeding 80% at a concentration of 0.3 nM. For example, B207S-DV29P achieved an inhibition rate of 93.37%, which is 23.59% higher than that of the positive control.

[0362] Table 3. DV29P-modified sequences with higher inhibition rates for HBsAg compared to the positive control

Sequence code	HBsAg inhibition rate (%)	Relative inhibition rate (positive control set as 1)	Increase% over positive control
B207S-DV29P	93.37	1.34	23.59
B1575-DV29P	88.62	1.27	18.84
B1572-DV29P	80.95	1.16	11.17
B418S-DV29P	80.25	1.15	10.47
A261-DV29P	73.97	1.06	4.19
A206-DV29P	73.97	1.06	4.19
A1550-DV29P	73.97	1.06	4.19
A1548-DV29P	72.57	1.04	2.79
A1573-DV29P	71.87	1.03	2.09
A1260-DV29P	71.87	1.03	2.09

A416-DV29P	71.18	1.02	1.4
A203-DV29P	70.48	1.01	0.7
A1547-DV29P	70.48	1.01	0.7
APC-VIR	69.78	1	0

[0363] (2) Sequences exhibiting inferior inhibition of HBsAg compared to the positive control are shown in Table 4. For example, the sequence B416S-DV29P showed an HBsAg inhibition rate of only 6.98%.

[0364] Table 4. DV29P-modified sequences with lower inhibition rates for HBsAg compared to the positive control

Sequence code	HBsAg inhibition rate (%)	Relative inhibition rate (positive control set as 1)
APC-VIR	69.78	1
A205-DV29P	68.38	0.98
A200-DV29P	66.99	0.96
A259-DV29P	58.62	0.84
A734-DV29P	57.22	0.82
A255-DV29P	57.22	0.82
A256-DV29P	54.43	0.78
A258-DV29P	54.43	0.78
A248-DV29P	54.43	0.78
B264-DV29P	51.64	0.74
B1560-DV29P	47.45	0.68
B208-DV29P	46.75	0.67
A1266-DV29P	45.36	0.65
B262-DV29P	43.96	0.63
B1556-DV29P	42.57	0.61
A413-DV29P	38.38	0.55
A463-DV29P	38.38	0.55
A536-DV29P	36.98	0.53
A707-DV29P	31.40	0.45
A1518-DV29P	21.63	0.31
A1520-DV29P	14.65	0.21
B416S-DV29P	6.98	0.1
A194-DV29P	-4.19	-0.06
A381-DV29P	-6.98	-0.1

[0365] (3) Sequences exhibiting superior inhibition of HBeAg compared to the positive control are shown in Table 5. Among these, B1575-DV29P, B207S-DV29P, B1572-DV29P, and B418S-DV29P ranked the highest, outperforming the positive control by 38.62%, 22.07%, 17.10%, and 13.24%, respectively.

[0366] Table 5. DV29P-modified sequences with higher inhibition rates for HBeAg compared to the positive control

Sequence code	HBeAg inhibition rate (%)	Relative inhibition rate (positive control set as 1)	Increase% over positive control
B1575-DV29P	66.21	2.4	38.62
B207S-DV29P	49.66	1.8	22.07
B1572-DV29P	44.69	1.62	17.10
B418S-DV29P	40.83	1.48	13.24
A1550-DV29P	39.45	1.43	11.86
A536-DV29P	38.35	1.39	10.76
A206-DV29P	37.24	1.35	9.65
A416-DV29P	35.04	1.27	7.45
A1573-DV29P	34.76	1.26	7.17
A381-DV29P	32.83	1.19	5.24
A1548-DV29P	32.55	1.18	4.96
A1260-DV29P	32	1.16	4.41
A1547-DV29P	32	1.16	4.41
A734-DV29P	31.17	1.13	3.58
A463-DV29P	30.07	1.09	2.48
A205-DV29P	29.79	1.08	2.20
A261-DV29P	28.42	1.03	0.83
APC-VIR	27.59	1	0

[0367] (4) Sequences exhibiting inferior inhibition of HBeAg compared to the positive control are shown in Table 6. For example, the sequence A1520-DV29P showed an inhibition rate of only 0.28% at a concentration of 0.3 nM.

[0368] Table 6. DV29P-modified sequences with lower inhibition rates for HBeAg compared to the positive control

Sequence code	HBeAg inhibition rate (%)	Relative inhibition rate (positive control set as 1)
APC-VIR	27.59	1
A200-DV29P	25.66	0.93

A203-DV29P	25.38	0.92
B208-DV29P	25.38	0.92
A255-DV29P	23.45	0.85
A413-DV29P	22.90	0.83
A258-DV29P	19.59	0.71
A256-DV29P	19.04	0.69
A1266-DV29P	16.83	0.61
A707-DV29P	16.28	0.59
B1560-DV29P	15.72	0.57
B262-DV29P	15.45	0.56
B1556-DV29P	14.35	0.52
A259-DV29P	12.14	0.44
B264-DV29P	11.59	0.42
A248-DV29P	9.66	0.35
B416S-DV29P	9.66	0.35
A1518-DV29P	4.14	0.15
A194-DV29P	2.21	0.08
A1520-DV29P	0.28	0.01

[0369] (ii) IC₅₀ experiment and cytotoxicity results

[0370] Nine sequences that demonstrated excellent performance in the single-concentration experiment, including B207S-DV29P, B1572-DV29P, B1575-DV29P, B418S-DV29P, A206-DV29P, A416-DV29P, A1548-DV29P, A1550-DV29P, and A1573-DV29P, were selected for IC₅₀ experiments. The results showed that the IC₅₀ values of these candidate sequences for both HBsAg and HBeAg were lower than those of the positive control, confirming their superior inhibitory effects. For HBsAg, A261-DV29P, A1573-DV29P, and A206-DV29P had IC₅₀ values of 0.019 nM, 0.022 nM, and 0.024 nM, respectively. For HBeAg, A1573-DV29P, B207S-DV29P, A206-DV29P, and A1550-DV29P had IC₅₀ values of 0.079 nM, 0.093 nM, 0.115 nM, and 0.138 nM, respectively. Detailed results are shown in Table 7.

[0371] The cytotoxicity results showed that among these sequences, except for sequence A416-DV29P, which exhibited a cell viability of 81.82% at the highest tested concentration of 10 nM, all other sequences demonstrated cell viabilities greater than 90%, indicating no significant cytotoxicity. This suggests that the sequences have a wide concentration range for selection. Detailed results are shown in Table 7.

[0372] Table 7: IC₅₀ experiment results for HBsAg and HBeAg

Sequence code	IC50 (nM)	IC50 (nM)	CC50 (nM)	Cell viability at 10 nM (%)
	HBsAg	HBeAg		
A261-DV29P	0.019	0.466	>10	120.22
A1573-DV29P	0.022	0.079	>10	91.90
A1550-DV29P	0.024	0.138	>10	117.93
A206-DV29P	0.026	0.115	>10	121.59
B207S-DV29P	0.027	0.093	>10	107.19
A416-DV29P	0.040	0.146	>10	81.82
A1260-DV29P	0.054	0.243	>10	107.40
B1575-DV29P	0.062	0.283	>10	93.09
A1548-DV29P	0.076	0.570	>10	108.60
APC-VIR	0.168	0.936	>10	101.21

[0373] Example 3: Inhibition of HBV gene expression by unmodified sequences

[0374] In this example, unmodified sequences corresponding to the modified sequences in Example 1 were synthesized and transfected into HepG2.2.15 cells using lipid nanoparticles (LNPs). The inhibitory effects of these sequences on HBsAg and HBeAg were detected using ELISA technology to screen for unmodified siRNA sequences with favorable inhibitory effects.

[0375] 1. Experimental materials

[0376] Test samples:

[0377] Unmodified small interfering RNA (siRNA) sequences listed in Table 8. All sequences were synthesized according to the methods described in Example 1.

[0378] Table 8: Unmodified siRNA sequences

Sequence code	Sense strand sequence 5'-3'	Sequence ID No	Antisense strand sequence 5'-3'	Sequence ID No
B207S	GGGGUUUUUCUUGUUGAC AA	1	UUGUCAACAAGAAAAACCC CGC	8
A1550	CCCGUCUGUGCCUUCUCA UCU	2	AGAUGAGAAGGCACAGACG GGGA	9
B1572	CCGGACCGUGUGCACUUC GCU	3	AGCGAAGUGCACACGGUCC GGCA	10
B1575	GACCGUGUGCACUUCGCU UCA	4	UGAAGCGAAGUGCACACGG UCCG	11
A1573	CGGACCGUGUGCACUUCG CUU	5	AAGCGAAGUGCACACGGUC CGGC	12
B418S	CUGCUAUGCCUCAUCUUC UU	6	AAGAAGAUGAGGCAUAGCA GCA	13

A416	UGCUGCUAUGCCUCAUCU UCU	7	AGAAGAUGAGGCAUAGCAG CAGG	14
A194	UCGUGUUACAGGCGGGGU UUU	15	AAAACCCCGCCUGAACACG AGA	47
A200	UACAGGCGGGGUUUUUCU UGU	16	ACAAGAAAAACCCCGCCUGU AAC	48
A203	AGGCGGGGUUUUUCUUGU UGA	17	UCAACAAGAAAAACCCCGCC UGU	49
A205	GCGGGGUUUUUCUUGUUG ACA	18	UGUCAACAAGAAAAACCCC GCCU	50
A206	CGGGGUUUUUCUUGUUGA CAA	19	UUGUCAACAAGAAAAACCC CGCC	51
A248	GUCUAGACUCGUGGUGGA CUU	20	AAGUCCACCACGAGUCUAG ACUC	52
A255	CUCGUGGUGGACUUCUCU CAA	21	UUGAGAGAAGUCCACCACG AGUC	53
A256	UCGUGGUGGACUUCUCUC AAU	22	AUUGAGAGAAGUCCACCAC GAGU	54
A258	GUGGUGGACUUCUCUCAA UUU	23	AAAUUGAGAGAAGUCCACC ACGA	55
A259	UGGUGGACUUCUCUCAAU UUU	24	AAAAUUGAGAGAAGUCCAC CACG	56
A261	GUGGACUUCUCUCAAUUU UCU	25	AGAAAAUUGAGAGAAGUCC ACCA	57
A381	GUGUCUGCGGCGUUUUAU CAU	26	AUGAUAAAACGCCGCAGAC ACAU	58
A413	UCCUGCUGCUAUGCCUCA UCU	27	AGAUGAGGCAUAGCAGCAG GAUG	59
A463	AUGUUGCCCGUUUGUCCU CUA	28	UAGAGGACAAACGGGCAAC AUAC	60
A536	CUGCUC AAGGAACCUCUA UGU	29	ACAUAGAGGUUCCUUGAGC AGUA	61
A707	UAGGGCUUUCUUUUACUG UUU	30	AAACAGUGGGGGAAAGCCC UACG	62
A734	CAGUUAUAUGGAUGAUGU GGU	31	ACCACAUCAUCCAUAUAACU GAA	63
A1260	CUGCCGAUCCAUAUGCG GAA	32	UUCCGCAGUAUGGAUCGGC AGAG	64
A1266	AUCCAUAUGCGGAACUC CUA	33	UAGGAGUUCCGCAGUAUGG AUCG	65
A1518	ACCACGGGGCGCACCUCU CUU	34	AAGAGAGGUGCGCCCCGUG GUCG	66

A1520	CACGGGGCGCACCUCUCU UUA	35	UAAAGAGAGGUGCGCCCCG UGGU	67
A1547	CUCCCCGUCUGUGCCUUC UCA	36	UGAGAAGGCACAGACGGGG AGUC	68
A1548	UCCCCGUCUGUGCCUUCU CAU	37	AUGAGAAGGCACAGACGGG GAGU	69
B208	GGGUUUUUCUUGUUGACA AGA	38	UCUUGUCAACAAGAAAAAC CCCG	70
B262	UGGACUUCUCUCAAUUUU CUA	39	UAGAAAAUUGAGAGAAGUC CACG	71
B264	GACUUCUCUCAAUUUUCU AGA	40	UCUAGAAAAUUGAGAGAAG UCCA	72
B1556	UGUGCCUUCUCAUCUGCC GGA	41	UCCGGCAGAUAGAGAAGGCA CAGA	73
B1560	CCUUCUCAUCUGCCGGAC CGU	42	ACGGUCCGGCAGAUAGAGAA GGCA	74
B416S	UGCUGCUAUGCCUCAUCU UA	43	UAAGAUGAGGCAUAGCAGC AGG	75
APC	GUGUGCACUUCGCUUCAC A	45	UGUGAAGCGAAGUGCACAC UU	79
ANC	GGUCUCUUCUAUCGAUCU UAU	46	AUAAGAUCGAUAGAAGAGA CCCA	80

[0379] Cell line: HepG2.2.15 cells.

[0380] Vehicle reagents: Sterile nuclease-free water and Gibco™ Opti-MEM™.

[0381] 2. Experimental methods

[0382] Referring to Example 2, the screening concentration was set at 0.3 nM.

[0383] 3. Experimental results

[0384] The experimental results demonstrated that the sequences B207S, B1572, B1575, B418S, A206, A416, A1548, A1550, and A1573 exhibited significantly superior inhibitory effects on HBsAg compared to other sequences, as well as significantly better inhibitory effects on HBeAg compared to other sequences.

[0385] Sequences exhibiting superior inhibition rates of HBsAg and HBeAg at a single concentration compared to the positive control are shown in Table 9, including B207S, B1575, B418S, A1573, B1572, A261, A206, A1550, A416, and A1548. Among these, B207S achieved inhibition rates of 77.02% for HBsAg and 40.74% for HBeAg, significantly outperforming the positive control sequence APC, which showed inhibition rates of 61.45%

for HBsAg and 20.85% for HBeAg.

[0386] Table 9: unmodified sequences with higher inhibition rates for HBsAg and HBeAg compared to the positive control

Sequence code	HBsAg inhibition rate (%)	HBeAg inhibition rate (%)
B207S	77.02	40.74
B1575	73.89	45.03
B418S	69.64	34.13
A1573	66.81	23.74
B1572	66.31	36.42
A261	66.26	22.06
A206	65.50	31.79
A1550	65.57	27.08
A416	63.78	24.35
A1548	62.62	27.22
APC	61.45	20.85

[0387] Example 4: Inhibition of HBsAg and HBeAg by sequences modified with different templates

[0388] In this example, 15 unmodified sequences—A205, B208, B207S, A261, B262, B264, A1550, B1556, B1560, B1575, A1573, B1572, B418S, A416, and B416S—were modified using 8 templates designed in the present disclosure (DV25P, DV26P, DV27P, DV28P, DV29P, DV32P, DV33P, and DV34P) as well as templates disclosed in prior art (DV30P, DV31P, DV21P, and DV22P), resulting in a total of 154 modified sequences. These sequences were transfected into HepG2.2.15 cells using lipid nanoparticles (LNPs), and their inhibitory effects on HBsAg and HBeAg were evaluated using ELISA technology.

[0389] 1. Experimental materials

[0390] Test Samples: Sequences obtained by modifying 15 siRNA unmodified sequences using different templates (DV25P, DV26P, DV27P, DV28P, DV29P, DV32P, DV33P, DV34P, DV30P, DV31P, DV21P, and DV22P) are shown in Table 11. The synthesis methods followed those described in Example 1.

[0391] Cell line: HepG2.2.15 cells.

[0392] Vehicle reagents: Sterile nuclease-free water and Gibco™ Opti-MEM.

[0393] The modification principles for the disclosed templates are as follows:

[0394] When the antisense strand is 23 nucleotides in length, the antisense strand is modified using one or a combination of two or more modification patterns as shown in Table 10-1:

[0395] Table 10-1 Antisense strand modification patterns

N o .	Antisense strand (AS) 5'-3'																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
A	2'- O Me + P S + E V P	2'- - F + P S	2'- O Me	2'- F	2'- - F	2'- - F	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - F	2'- - O Me	2'- - F	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- O Me +P S	2'- - O Me + P S	2'- - O Me
B	2'- O Me + P S + E V P	2'- - F + P S	2'- F	2'- F	2'- - O Me	2'- - F	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - F	2'- - O Me	2'- - F	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- O Me +P S	2'- - O Me + P S	2'- - O Me
C	2'- O Me + P S + E V P	2'- - F + P S	2'- O Me	2'- O Me	2'- - F	2'- - F	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- - F	2'- - O Me	2'- - F	2'- - O Me	2'- - O Me	2'- - O Me	2'- - O Me	2'- O Me +P S	2'- - O Me + P S	2'- - O Me
D	2'	2	2	2	2'	2	2'	2'	2'	2'	2'	2'	2'	2	2'	2	2'	2'	2'	2'	2'-	2'	2'

	- O M e + P S + E V P	' - F + P S	' - F	' - O M e	- O M e	' - F M e	- O M e	- O M e	- O M e	- O M e	- O M e	- O M e	- O M e	' - F M e	- O M e	' - F	- O M e	- O M e	- O M e	- O M e	O Me +P S	- O M e + P S	- O M e
E	2' - O M e + P S + E V P	2' - F + P S	2' - O M e	2' - O M e	2' - O M e	2' - F M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F M e	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O Me +P S	2' - O M e + P S	2' - O M e
F	2' - O M e + P S + E V P	2' - F + P S	2' - O M e	2' - O M e	2' - O M e	2' - F M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F M e	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O Me +P S	2' - O M e + P S	2' - O M e

[0396] When the antisense strand is 22 nucleotides in length, the antisense strand is modified using one or a combination of two or more modification patterns as shown in Table 10-2:

[0397] Table 10-2 Antisense strand modification patterns

N o .	Antisense strand (AS) 5'-3'																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A	2'	2	2	2	2'	2	2'	2'	2'	2'	2'	2'	2'	2	2'	2	2'	2'	2'	2'	2'

	- O Me + P S + E V P	' F + P S	' O Me	' F	- F	' - F	- O Me	- O Me	- O Me	- O Me	- O Me	- O Me	- O Me	' - F	- O Me	' - F	- O Me	- O Me	- O Me	O Me + P S	- O Me + P S	- O Me
B	2' - O Me + P S + E V P	2' F + P S	2' F	2' F	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - F	2' - O Me	2' - F	2' - O Me	2' - O Me	2' - O Me	2' - O Me + P S	2' - O Me + P S	2' - O Me
C	2' - O Me + P S + E V P	2' F + P S	2' O Me	2' O Me	2' - F	2' - F	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - F	2' - O Me	2' - F	2' - O Me	2' - O Me	2' - O Me	2' - O Me + P S	2' - O Me + P S	2' - O Me
D	2' - O Me + P S +	2' F + P S	2' F	2' O Me	2' - O Me	2' - F	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - O Me	2' - F	2' - O Me	2' - F	2' - O Me	2' - O Me	2' - O Me	2' - O Me + P S	2' - O Me + P S	2' - O Me

	E V P																					
E	2'- - O M e + P S + E V P	2' - F + P S	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2'- O Me +P S	2' - O M e + P S	2' - O M e
F	2'- - O M e + P S + E V P	2' - F + P S	2' - F	2' - O M e	2' - F	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2'- O Me +P S	2' - O M e + P S	2' - O M e

[0398] When the sense strand is 21 nucleotides in length, the sense strand is modified using one or a combination of two modification patterns as shown in Table 10-3:

[0399] Table 10-3 Sense strand modification patterns

N o .	Sense strand (SS) 5'-3'																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
a	2'- O M e + P S	2'- O M e + P S	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- F	2'- O M e	2'- F	2'- F	2'- F	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- F	2'- O M e	2'- O M e	2'- O M e	2'- O M e
b	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O	2'- O

	M e + P S	M e + P S	M e	M e	M e	M e	- F	M e	- F	M e	- F	M e	M e	M e	M e	M e	- F	M e	M e	M e	M e
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[0400] When the sense strand is 20 nucleotides in length, the sense strand is modified using one or a combination of two modification patterns as shown in Table 10-4:

[0401] Table 10-4 Sense strand modification patterns

N o .	Sense strand (SS) 5'-3'																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
a	2'- O M e +P S	2'- O M e +P S	2'- O M e	2'- O M e	2'- O M e	2'- O M e - F	2'- O M e	2'- O M e - F	2'- O M e - F	2'- O M e - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e
b	2'- O M e +P S	2'- O M e +P S	2'- O M e	2'- O M e	2'- O M e	2'- O M e - F	2'- O M e	2'- O M e - F	2'- O M e - F	2'- O M e - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e

[0402] In Tables 10-1 to 10-4, 2'-OMe represents 2'-methoxy; 2'-F represents 2'-fluoro; PS represents phosphorothioate backbone; EVP represents 5'-(E)-vinylphosphonate;

[0403] the siRNA modification template with antisense strand modified by pattern A and sense strand modified by pattern a is designated as DV27P;

[0404] the siRNA modification template with antisense strand modified by pattern A and sense strand modified by pattern b is designated as DV29P;

[0405] the siRNA modification template with antisense strand modified by pattern B and sense strand modified by pattern a is designated as DV26P;

[0406] the siRNA modification template with antisense strand modified by pattern B and sense strand modified by pattern b is designated as DV28P;

[0407] the siRNA modification template with antisense strand modified by pattern C and sense strand modified by pattern b is designated as DV32P;

[0408] the siRNA modification template with antisense strand modified by pattern D and sense strand modified by pattern b is designated as DV34P;

[0409] the siRNA modification template with antisense strand modified by pattern E and sense strand modified by pattern a is designated as DV25P;

[0410] the siRNA modification template with antisense strand modified by pattern F and sense strand modified by pattern b is designated as DV33P.

[0411] The synthesis methods for each sequence are the same as in Example 1.

[0412] Table 11: Sequences modified with different modification templates

Unmodified sequence code	Sequence code	Sense strand sequence 5'-3'	Sequence ID No	Unmodified sequence ID No	Antisense strand sequence 5'-3'	Sequence ID No	Unmodified sequence ID No
B207S	B20 7S- DV2 9P	Gms-Gms-Gm-Gm-Um-Uf- Um-Uf-Um-Cf-Um-Um-Gm- Um-Um-Gf-Am-Cm-Am-Am	81	1	UmsEVP-Ufs-Gm-Uf-Cf-Af-Am- Cm-Am-Am-Gm-Am-Am-Af-Am- Af-Cm-Cm-Cm-Cms-Gms-Cm	179	8
B207S	B20 7S- DV2 6P	Gms-Gms-Gm-Gm-Um-Uf- Um-Uf-Uf-Cf-Um-Um-Gm- Um-Um-Gf-Am-Cm-Am-Am	82	1	UmsEVP-Ufs-Gf-Uf-Cm-Af-Am- Cm-Am-Am-Gm-Am-Am-Af-Am- Af-Cm-Cm-Cm-Cms-Gms-Cm	180	8
B207S	B20 7S- DV2 7P	Gms-Gms-Gm-Gm-Um-Uf- Um-Uf-Uf-Cf-Um-Um-Gm- Um-Um-Gf-Am-Cm-Am-Am	82	1	UmsEVP-Ufs-Gm-Uf-Cf-Af-Am- Cm-Am-Am-Gm-Am-Am-Af-Am- Af-Cm-Cm-Cm-Cms-Gms-Cm	179	8
B207S	B20 7S- DV2 8P	Gms-Gms-Gm-Gm-Um-Uf- Um-Uf-Um-Cf-Um-Um-Gm- Um-Um-Gf-Am-Cm-Am-Am	81	1	UmsEVP-Ufs-Gf-Uf-Cm-Af-Am- Cm-Am-Am-Gm-Am-Am-Af-Am- Af-Cm-Cm-Cm-Cms-Gms-Cm	180	8
B207S	B20 7S- DV3 2P	Gms-Gms-Gm-Gm-Um-Uf- Um-Uf-Um-Cf-Um-Um-Gm- Um-Um-Gf-Am-Cm-Am-Am	81	1	UmsEVP-Ufs-Gm-Um-Cf-Af-Am- Cm-Am-Am-Gm-Am-Am-Af-Am- Af-Cm-Cm-Cm-Cms-Gms-Cm	181	8
B207S	B20 7S- DV3 4P	Gms-Gms-Gm-Gm-Um-Uf- Um-Uf-Um-Cf-Um-Um-Gm- Um-Um-Gf-Am-Cm-Am-Am	81	1	UmsEVP-Ufs-Gf-Um-Cm-Af-Am- Cm-Am-Am-Gm-Am-Am-Af-Am- Af-Cm-Cm-Cm-Cms-Gms-Cm	182	8
B207S	B20 7S- DV2 5P	Gms-Gms-Gm-Gm-Um-Uf- Um-Uf-Uf-Cf-Um-Um-Gm- Um-Um-Gf-Am-Cm-Am-Am	82	1	UmsEVP-Ufs-Gm-Um-Cm-Af-Am- Cm-Am-Am-Gm-Am-Am-Af-Am- Af-Cm-Cm-Cm-Cms-Gms-Cm	183	8

B207S	B20 7S- DV3 3P	Gms-Gms-Gm-Gm-Um-Uf- Um-Uf-Um-Cf-Um-Um-Gm- Um-Um-Gf-Am-Cm-Am-Am	81	1	UmsEVP-Ufs-Gf-Um-Cf-Af-Am- Cm-Am-Am-Gm-Am-Am-Af-Am- Af-Cm-Cm-Cm-Cms-Gms-Cm	184	8
B207S	B20 7S- DV3 0P	Gms-Gms-Gm-Gm-Um-Uf- Um-Uf-Uf-Cf-Um-Um-Gm- Um-Um-Gm-Am-Cm-Am-Am	83	1	UmsEVP-Ufs-Gm-Um-Cm-Af-Am- Cm-Am-Am-Gm-Am-Am-Af-Am- Am-Cm-Cm-Cm-Cms-Gms-Cm	185	8
B207S	B20 7S- DV3 1P	Gms-Gms-Gm-Gm-Um-Uf- Um-Uf-Uf-Cm-Um-Um-Gm- Um-Um-Gm-Am-Cm-Am-Am	84	1	UmsEVP-Ufs-Gm-Um-Cm-Af-Am- Cm-Am-Am-Gm-Am-Am-Af-Am- Af-Cm-Cm-Cm-Cms-Gms-Cm	186	8
B207S	B20 7S- DV2 1P	Gms-Gms-Gm-Gm-Um-Uf- Um-Uf-Uf-Cf-Um-Um-Gm- Um-Uf-Gf-Am-Cm-Am-Am	85	1	UmsEVP-Ufs-Gm-Um-Cm-Af-Am- Cm-Am-Am-Gm-Am-Am-Af-Am- Af-Cm-Cm-Cm-Cms-Gms-Cm	186	8
B207S	B20 7S- DV2 2P	Gms-Gms-Gm-Gm-Um-Uf- Um-Uf-Uf-Cf-Um-Um-Gm- Um-Um-Gm-Am-Cm-Am-Am	83	1	UmsEVP-Ufs-Gm-Um-Cm-Af-Am- Cm-Am-Am-Gm-Am-Am-Af-Am- Af-Cm-Cm-Cm-Cms-Gms-Cm	186	8
A1550	A15 50- DV2 9P	Cms-Cms-Cm-Gm-Um-Cm- Uf-Gm-Uf-Gm-Cf-Cm-Um- Um-Cm-Um-Cf-Am-Um-Cm- Um	86	2	AmsEVP-Gfs-Am-Uf-Gf-Af-Gm- Am-Am-Gm-Gm-Cm-Am-Cf-Am- Gf-Am-Cm-Gm-Gm-Gms-Gms-Am	187	9
A1550	B15 50- DV2 6P	Cms-Cms-Cm-Gm-Um-Cm- Uf-Gm-Uf-Gf-Cf-Cm-Um- Um-Cm-Um-Cf-Am-Um-Cm- Um	87	2	AmsEVP-Gfs-Af-Uf-Gm-Af-Gm- Am-Am-Gm-Gm-Cm-Am-Cf-Am- Gf-Am-Cm-Gm-Gm-Gms-Gms-Am	188	9
A1550	B15 50- DV2 7P	Cms-Cms-Cm-Gm-Um-Cm- Uf-Gm-Uf-Gf-Cf-Cm-Um- Um-Cm-Um-Cf-Am-Um-Cm- Um	87	2	AmsEVP-Gfs-Am-Uf-Gf-Af-Gm- Am-Am-Gm-Gm-Cm-Am-Cf-Am- Gf-Am-Cm-Gm-Gm-Gms-Gms-Am	187	9
A1550	B15 50- DV2 8P	Cms-Cms-Cm-Gm-Um-Cm- Uf-Gm-Uf-Gm-Cf-Cm-Um- Um-Cm-Um-Cf-Am-Um-Cm- Um	86	2	AmsEVP-Gfs-Af-Uf-Gm-Af-Gm- Am-Am-Gm-Gm-Cm-Am-Cf-Am- Gf-Am-Cm-Gm-Gm-Gms-Gms-Am	188	9
A1550	B15 50- DV3 2P	Cms-Cms-Cm-Gm-Um-Cm- Uf-Gm-Uf-Gm-Cf-Cm-Um- Um-Cm-Um-Cf-Am-Um-Cm- Um	86	2	AmsEVP-Gfs-Am-Um-Gf-Af-Gm- Am-Am-Gm-Gm-Cm-Am-Cf-Am- Gf-Am-Cm-Gm-Gm-Gms-Gms-Am	189	9
A1550	B15 50- DV3 4P	Cms-Cms-Cm-Gm-Um-Cm- Uf-Gm-Uf-Gm-Cf-Cm-Um- Um-Cm-Um-Cf-Am-Um-Cm- Um	86	2	AmsEVP-Gfs-Af-Um-Gm-Af-Gm- Am-Am-Gm-Gm-Cm-Am-Cf-Am- Gf-Am-Cm-Gm-Gm-Gms-Gms-Am	190	9

A1550	B15 50- DV2 5P	Cms-Cms-Cm-Gm-Um-Cm- Uf-Gm-Uf-Gf-Cf-Cm-Um- Um-Cm-Um-Cf-Am-Um-Cm- Um	87	2	AmsEVP-Gfs-Am-Um-Gm-Af-Gm- Am-Am-Gm-Gm-Cm-Am-Cf-Am- Gf-Am-Cm-Gm-Gm-Gms-Gms-Am	191	9
A1550	B15 50- DV3 3P	Cms-Cms-Cm-Gm-Um-Cm- Uf-Gm-Uf-Gm-Cf-Cm-Um- Um-Cm-Um-Cf-Am-Um-Cm- Um	86	2	AmsEVP-Gfs-Af-Um-Gf-Af-Gm- Am-Am-Gm-Gm-Cm-Am-Cf-Am- Gf-Am-Cm-Gm-Gm-Gms-Gms-Am	192	9
A1550	B15 50- DV3 0P	Cms-Cms-Cm-Gm-Um-Cm- Uf-Gm-Uf-Gf-Cf-Cm-Um- Um-Cm-Um-Cm-Am-Um- Cm-Um	88	2	AmsEVP-Gfs-Am-Um-Gm-Af-Gm- Am-Am-Gm-Gm-Cm-Am-Cf-Am- Gm-Am-Cm-Gm-Gm-Gms-Gms- Am	193	9
A1550	B15 50- DV3 1P	Cms-Cms-Cm-Gm-Um-Cm- Uf-Gm-Uf-Gf-Cm-Cm-Um- Um-Cm-Um-Cm-Am-Um- Cm-Um	89	2	AmsEVP-Gfs-Am-Um-Gm-Af-Gm- Am-Am-Gm-Gm-Cm-Am-Cf-Am- Gf-Am-Cm-Gm-Gm-Gms-Gms-Am	194	9
A1550	B15 50- DV2 1P	Cms-Cms-Cm-Gm-Um-Cm- Uf-Gm-Uf-Gf-Cf-Cm-Um- Um-Cm-Uf-Cf-Am-Um-Cm- Um	90	2	AmsEVP-Gfs-Am-Um-Gm-Af-Gm- Am-Am-Gm-Gm-Cm-Am-Cf-Am- Gf-Am-Cm-Gm-Gm-Gms-Gms-Am	194	9
A1550	B15 50- DV2 2P	Cms-Cms-Cm-Gm-Um-Cm- Uf-Gm-Uf-Gf-Cf-Cm-Um- Um-Cm-Um-Cm-Am-Um- Cm-Um	88	2	AmsEVP-Gfs-Am-Um-Gm-Af-Gm- Am-Am-Gm-Gm-Cm-Am-Cf-Am- Gf-Am-Cm-Gm-Gm-Gms-Gms-Am	194	9
B1572	B15 72- DV2 9P	Cms-Cms-Gm-Gm-Am-Cm- Cf-Gm-Uf-Gm-Uf-Gm-Cm- Am-Cm-Um-Uf-Cm-Gm-Cm- Um	91	3	AmsEVP-Gfs-Cm-Gf-Af-Af-Gm- Um-Gm-Cm-Am-Cm-Am-Cf-Gm- Gf-Um-Cm-Cm-Gm-Gms-Cms-Am	195	10
B1572	B15 72- DV2 6P	Cms-Cms-Gm-Gm-Am-Cm- Cf-Gm-Uf-Gf-Uf-Gm-Cm- Am-Cm-Um-Uf-Cm-Gm-Cm- Um	92	3	AmsEVP-Gfs-Cf-Gf-Am-Af-Gm- Um-Gm-Cm-Am-Cm-Am-Cf-Gm- Gf-Um-Cm-Cm-Gm-Gms-Cms-Am	196	10
B1572	B15 72- DV2 7P	Cms-Cms-Gm-Gm-Am-Cm- Cf-Gm-Uf-Gf-Uf-Gm-Cm- Am-Cm-Um-Uf-Cm-Gm-Cm- Um	92	3	AmsEVP-Gfs-Cm-Gf-Af-Af-Gm- Um-Gm-Cm-Am-Cm-Am-Cf-Gm- Gf-Um-Cm-Cm-Gm-Gms-Cms-Am	195	10
B1572	B15 72- DV2 8P	Cms-Cms-Gm-Gm-Am-Cm- Cf-Gm-Uf-Gm-Uf-Gm-Cm- Am-Cm-Um-Uf-Cm-Gm-Cm- Um	91	3	AmsEVP-Gfs-Cf-Gf-Am-Af-Gm- Um-Gm-Cm-Am-Cm-Am-Cf-Gm- Gf-Um-Cm-Cm-Gm-Gms-Cms-Am	196	10
B1572	B15 72- DV3 2P	Cms-Cms-Gm-Gm-Am-Cm- Cf-Gm-Uf-Gm-Uf-Gm-Cm- Am-Cm-Um-Uf-Cm-Gm-Cm- Um	91	3	AmsEVP-Gfs-Cm-Gm-Af-Af-Gm- Um-Gm-Cm-Am-Cm-Am-Cf-Gm- Gf-Um-Cm-Cm-Gm-Gms-Cms-Am	197	10

B1572	B15 72- DV3 4P	Cms-Cms-Gm-Gm-Am-Cm- Cf-Gm-Uf-Gm-Uf-Gm-Cm- Am-Cm-Um-Uf-Cm-Gm-Cm- Um	91	3	AmsEVP-Gfs-Cf-Gm-Am-Af-Gm- Um-Gm-Cm-Am-Cm-Am-Cf-Gm- Gf-Um-Cm-Cm-Gm-Gms-Cms-Am	198	10
B1572	B15 72- DV3 0P	Cms-Cms-Gm-Gm-Am-Cm- Cf-Gm-Uf-Gf-Uf-Gm-Cm- Am-Cm-Um-Um-Cm-Gm- Cm-Um	93	3	AmsEVP-Gfs-Cm-Gm-Am-Af-Gm- Um-Gm-Cm-Am-Cm-Am-Cf-Gm- Gm-Um-Cm-Cm-Gm-Gms-Cms- Am	199	10
B1572	B15 72- DV3 1P	Cms-Cms-Gm-Gm-Am-Cm- Cf-Gm-Uf-Gf-Um-Gm-Cm- Am-Cm-Um-Um-Cm-Gm- Cm-Um	94	3	AmsEVP-Gfs-Cm-Gm-Am-Af-Gm- Um-Gm-Cm-Am-Cm-Am-Cf-Gm- Gf-Um-Cm-Cm-Gm-Gms-Cms-Am	200	10
B1572	B15 72- DV2 1P	Cms-Cms-Gm-Gm-Am-Cm- Cf-Gm-Uf-Gf-Uf-Gm-Cm- Am-Cm-Uf-Uf-Cm-Gm-Cm- Um	95	3	AmsEVP-Gfs-Cm-Gm-Am-Af-Gm- Um-Gm-Cm-Am-Cm-Am-Cf-Gm- Gf-Um-Cm-Cm-Gm-Gms-Cms-Am	200	10
B1572	B15 72- DV2 2P	Cms-Cms-Gm-Gm-Am-Cm- Cf-Gm-Uf-Gf-Uf-Gm-Cm- Am-Cm-Um-Um-Cm-Gm- Cm-Um	93	3	AmsEVP-Gfs-Cm-Gm-Am-Af-Gm- Um-Gm-Cm-Am-Cm-Am-Cf-Gm- Gf-Um-Cm-Cm-Gm-Gms-Cms-Am	200	10
B1575	B15 75- DV2 9P	Gms-Ams-Cm-Cm-Gm-Um- Gf-Um-Gf-Cm-Af-Cm-Um- Um-Cm-Gm-Cf-Um-Um-Cm- Am	96	4	UmsEVP-Gfs-Am-Af-Gf-Cf-Gm- Am-Am-Gm-Um-Gm-Cm-Af-Cm- Af-Cm-Gm-Gm-Um-Cms-Cms-Gm	201	11
B1575	B15 75- DV2 6P	Gms-Ams-Cm-Cm-Gm-Um- Gf-Um-Gf-Cf-Af-Cm-Um- Um-Cm-Gm-Cf-Um-Um-Cm- Am	97	4	UmsEVP-Gfs-Af-Af-Gm-Cf-Gm- Am-Am-Gm-Um-Gm-Cm-Af-Cm- Af-Cm-Gm-Gm-Um-Cms-Cms-Gm	202	11
B1575	B15 75- DV2 7P	Gms-Ams-Cm-Cm-Gm-Um- Gf-Um-Gf-Cf-Af-Cm-Um- Um-Cm-Gm-Cf-Um-Um-Cm- Am	97	4	UmsEVP-Gfs-Am-Af-Gf-Cf-Gm- Am-Am-Gm-Um-Gm-Cm-Af-Cm- Af-Cm-Gm-Gm-Um-Cms-Cms-Gm	201	11
B1575	B15 75- DV2 8P	Gms-Ams-Cm-Cm-Gm-Um- Gf-Um-Gf-Cm-Af-Cm-Um- Um-Cm-Gm-Cf-Um-Um-Cm- Am	96	4	UmsEVP-Gfs-Af-Af-Gm-Cf-Gm- Am-Am-Gm-Um-Gm-Cm-Af-Cm- Af-Cm-Gm-Gm-Um-Cms-Cms-Gm	202	11
B1575	B15 75- DV3 2P	Gms-Ams-Cm-Cm-Gm-Um- Gf-Um-Gf-Cm-Af-Cm-Um- Um-Cm-Gm-Cf-Um-Um-Cm- Am	96	4	UmsEVP-Gfs-Am-Am-Gf-Cf-Gm- Am-Am-Gm-Um-Gm-Cm-Af-Cm- Af-Cm-Gm-Gm-Um-Cms-Cms-Gm	203	11
B1575	B15 75- DV3 4P	Gms-Ams-Cm-Cm-Gm-Um- Gf-Um-Gf-Cm-Af-Cm-Um- Um-Cm-Gm-Cf-Um-Um-Cm- Am	96	4	UmsEVP-Gfs-Af-Am-Gm-Cf-Gm- Am-Am-Gm-Um-Gm-Cm-Af-Cm- Af-Cm-Gm-Gm-Um-Cms-Cms-Gm	204	11

B1575	B15 75- DV3 0P	Gms-Ams-Cm-Cm-Gm-Um- Gf-Um-Gf-Cf-Af-Cm-Um- Um-Cm-Gm-Cm-Um-Um- Cm-Am	98	4	UmsEVP-Gfs-Am-Am-Gm-Cf-Gm- Am-Am-Gm-Um-Gm-Cm-Af-Cm- Am-Cm-Gm-Gm-Um-Cms-Cms- Gm	205	11
B1575	B15 75- DV3 1P	Gms-Ams-Cm-Cm-Gm-Um- Gf-Um-Gf-Cf-Am-Cm-Um- Um-Cm-Gm-Cm-Um-Um- Cm-Am	99	4	UmsEVP-Gfs-Am-Am-Gm-Cf-Gm- Am-Am-Gm-Um-Gm-Cm-Af-Cm- Af-Cm-Gm-Gm-Um-Cms-Cms-Gm	206	11
B1575	B15 75- DV2 1P	Gms-Ams-Cm-Cm-Gm-Um- Gf-Um-Gf-Cf-Af-Cm-Um- Um-Cm-Gf-Cf-Um-Um-Cm- Am	100	4	UmsEVP-Gfs-Am-Am-Gm-Cf-Gm- Am-Am-Gm-Um-Gm-Cm-Af-Cm- Af-Cm-Gm-Gm-Um-Cms-Cms-Gm	206	11
B1575	B15 75- DV2 2P	Gms-Ams-Cm-Cm-Gm-Um- Gf-Um-Gf-Cf-Af-Cm-Um- Um-Cm-Gm-Cm-Um-Um- Cm-Am	98	4	UmsEVP-Gfs-Am-Am-Gm-Cf-Gm- Am-Am-Gm-Um-Gm-Cm-Af-Cm- Af-Cm-Gm-Gm-Um-Cms-Cms-Gm	206	11
A1573	A15 73- DV2 9P	Cms-Gms-Gm-Am-Cm-Cm- Gf-Um-Gf-Um-Gf-Cm-Am- Cm-Um-Um-Cf-Gm-Cm-Um- Um	101	5	AmsEVP-Afs-Gm-Cf-Gf-Af-Am- Gm-Um-Gm-Cm-Am-Cm-Af-Cm- Gf-Gm-Um-Cm-Cm-Gms-Gms-Cm	207	12
A1573	B15 73- DV2 6P	Cms-Gms-Gm-Am-Cm-Cm- Gf-Um-Gf-Uf-Gf-Cm-Am- Cm-Um-Um-Cf-Gm-Cm-Um- Um	102	5	AmsEVP-Afs-Gf-Cf-Gm-Af-Am- Gm-Um-Gm-Cm-Am-Cm-Af-Cm- Gf-Gm-Um-Cm-Cm-Gms-Gms-Cm	208	12
A1573	B15 73- DV2 7P	Cms-Gms-Gm-Am-Cm-Cm- Gf-Um-Gf-Uf-Gf-Cm-Am- Cm-Um-Um-Cf-Gm-Cm-Um- Um	102	5	AmsEVP-Afs-Gm-Cf-Gf-Af-Am- Gm-Um-Gm-Cm-Am-Cm-Af-Cm- Gf-Gm-Um-Cm-Cm-Gms-Gms-Cm	207	12
A1573	B15 73- DV2 8P	Cms-Gms-Gm-Am-Cm-Cm- Gf-Um-Gf-Um-Gf-Cm-Am- Cm-Um-Um-Cf-Gm-Cm-Um- Um	101	5	AmsEVP-Afs-Gf-Cf-Gm-Af-Am- Gm-Um-Gm-Cm-Am-Cm-Af-Cm- Gf-Gm-Um-Cm-Cm-Gms-Gms-Cm	208	12
A1573	B15 73- DV3 2P	Cms-Gms-Gm-Am-Cm-Cm- Gf-Um-Gf-Um-Gf-Cm-Am- Cm-Um-Um-Cf-Gm-Cm-Um- Um	101	5	AmsEVP-Afs-Gm-Cm-Gf-Af-Am- Gm-Um-Gm-Cm-Am-Cm-Af-Cm- Gf-Gm-Um-Cm-Cm-Gms-Gms-Cm	209	12
A1573	B15 73- DV3 4P	Cms-Gms-Gm-Am-Cm-Cm- Gf-Um-Gf-Um-Gf-Cm-Am- Cm-Um-Um-Cf-Gm-Cm-Um- Um	101	5	AmsEVP-Afs-Gf-Cm-Gm-Af-Am- Gm-Um-Gm-Cm-Am-Cm-Af-Cm- Gf-Gm-Um-Cm-Cm-Gms-Gms-Cm	210	12
A1573	B15 73- DV3 0P	Cms-Gms-Gm-Am-Cm-Cm- Gf-Um-Gf-Uf-Gf-Cm-Am- Cm-Um-Um-Cm-Gm-Cm- Um-Um	103	5	AmsEVP-Afs-Gm-Cm-Gm-Af-Am- Gm-Um-Gm-Cm-Am-Cm-Af-Cm- Gm-Gm-Um-Cm-Cm-Gms-Gms- Cm	211	12

A1573	B15 73- DV3 1P	Cms-Gms-Gm-Am-Cm-Cm- Gf-Um-Gf-Uf-Gm-Cm-Am- Cm-Um-Um-Cm-Gm-Cm- Um-Um	104	5	AmsEVP-Afs-Gm-Cm-Gm-Af-Am- Gm-Um-Gm-Cm-Am-Cm-Af-Cm- Gf-Gm-Um-Cm-Cm-Gms-Gms-Cm	212	12
A1573	B15 73- DV2 1P	Cms-Gms-Gm-Am-Cm-Cm- Gf-Um-Gf-Uf-Gf-Cm-Am- Cm-Um-Uf-Cf-Gm-Cm-Um- Um	105	5	AmsEVP-Afs-Gm-Cm-Gm-Af-Am- Gm-Um-Gm-Cm-Am-Cm-Af-Cm- Gf-Gm-Um-Cm-Cm-Gms-Gms-Cm	212	12
A1573	B15 73- DV2 2P	Cms-Gms-Gm-Am-Cm-Cm- Gf-Um-Gf-Uf-Gf-Cm-Am- Cm-Um-Um-Cm-Gm-Cm- Um-Um	103	5	AmsEVP-Afs-Gm-Cm-Gm-Af-Am- Gm-Um-Gm-Cm-Am-Cm-Af-Cm- Gf-Gm-Um-Cm-Cm-Gms-Gms-Cm	212	12
B418S	B41 8S- DV2 9P	Cms-Ums-Gm-Cm-Um-Af- Um-Gf-Cm-Cf-Um-Cm-Am- Um-Cm-Uf-Um-Cm-Um-Um	106	6	AmsEVP-Afs-Gm-Af-Af-Gf-Am- Um-Gm-Am-Gm-Gm-Cm-Af-Um- Af-Gm-Cm-Am-Gms-Cms-Am	213	13
B418S	B41 8S- DV2 6P	Cms-Ums-Gm-Cm-Um-Af- Um-Gf-Cf-Cf-Um-Cm-Am- Um-Cm-Uf-Um-Cm-Um-Um	107	6	AmsEVP-Afs-Gf-Af-Am-Gf-Am- Um-Gm-Am-Gm-Gm-Cm-Af-Um- Af-Gm-Cm-Am-Gms-Cms-Am	214	13
B418S	B41 8S- DV2 7P	Cms-Ums-Gm-Cm-Um-Af- Um-Gf-Cf-Cf-Um-Cm-Am- Um-Cm-Uf-Um-Cm-Um-Um	107	6	AmsEVP-Afs-Gm-Af-Af-Gf-Am- Um-Gm-Am-Gm-Gm-Cm-Af-Um- Af-Gm-Cm-Am-Gms-Cms-Am	213	13
B418S	B41 8S- DV2 8P	Cms-Ums-Gm-Cm-Um-Af- Um-Gf-Cm-Cf-Um-Cm-Am- Um-Cm-Uf-Um-Cm-Um-Um	106	6	AmsEVP-Afs-Gf-Af-Am-Gf-Am- Um-Gm-Am-Gm-Gm-Cm-Af-Um- Af-Gm-Cm-Am-Gms-Cms-Am	214	13
B418S	B41 8S- DV3 2P	Cms-Ums-Gm-Cm-Um-Af- Um-Gf-Cm-Cf-Um-Cm-Am- Um-Cm-Uf-Um-Cm-Um-Um	106	6	AmsEVP-Afs-Gm-Am-Af-Gf-Am- Um-Gm-Am-Gm-Gm-Cm-Af-Um- Af-Gm-Cm-Am-Gms-Cms-Am	215	13
B418S	B41 8S- DV3 4P	Cms-Ums-Gm-Cm-Um-Af- Um-Gf-Cm-Cf-Um-Cm-Am- Um-Cm-Uf-Um-Cm-Um-Um	106	6	AmsEVP-Afs-Gf-Am-Am-Gf-Am- Um-Gm-Am-Gm-Gm-Cm-Af-Um- Af-Gm-Cm-Am-Gms-Cms-Am	216	13
B418S	B41 8S- DV3 0P	Cms-Ums-Gm-Cm-Um-Af- Um-Gf-Cf-Cf-Um-Cm-Am- Um-Cm-Um-Um-Cm-Um-Um	108	6	AmsEVP-Afs-Gm-Am-Am-Gf-Am- Um-Gm-Am-Gm-Gm-Cm-Af-Um- Am-Gm-Cm-Am-Gms-Cms-Am	217	13
B418S	B41 8S- DV3 1P	Cms-Ums-Gm-Cm-Um-Af- Um-Gf-Cf-Cm-Um-Cm-Am- Um-Cm-Um-Um-Cm-Um-Um	109	6	AmsEVP-Afs-Gm-Am-Am-Gf-Am- Um-Gm-Am-Gm-Gm-Cm-Af-Um- Af-Gm-Cm-Am-Gms-Cms-Am	218	13

B418S	B41 8S- DV2 1P	Cms-Ums-Gm-Cm-Um-Af- Um-Gf-Cf-Cf-Um-Cm-Am- Um-Cf-Uf-Um-Cm-Um-Um	110	6	AmsEVP-Afs-Gm-Am-Am-Gf-Am- Um-Gm-Am-Gm-Gm-Cm-Af-Um- Af-Gm-Cm-Am-Gms-Cms-Am	218	13
B418S	B41 8S- DV2 2P	Cms-Ums-Gm-Cm-Um-Af- Um-Gf-Cf-Cf-Um-Cm-Am- Um-Cm-Um-Um-Cm-Um-Um	108	6	AmsEVP-Afs-Gm-Am-Am-Gf-Am- Um-Gm-Am-Gm-Gm-Cm-Af-Um- Af-Gm-Cm-Am-Gms-Cms-Am	218	13
A416	A41 6- DV2 9P	Ums-Gms-Cm-Um-Gm-Cm- Uf-Am-Uf-Gm-Cf-Cm-Um- Cm-Am-Um-Cf-Um-Um-Cm- Um	111	7	AmsEVP-Gfs-Am-Af-Gf-Af-Um- Gm-Am-Gm-Gm-Cm-Am-Uf-Am- Gf-Cm-Am-Gm-Cm-Ams-Gms-Gm	219	14
A416	B41 6- DV2 6P	Ums-Gms-Cm-Um-Gm-Cm- Uf-Am-Uf-Gf-Cf-Cm-Um- Cm-Am-Um-Cf-Um-Um-Cm- Um	112	7	AmsEVP-Gfs-Af-Af-Gm-Af-Um- Gm-Am-Gm-Gm-Cm-Am-Uf-Am- Gf-Cm-Am-Gm-Cm-Ams-Gms-Gm	220	14
A416	B41 6- DV2 7P	Ums-Gms-Cm-Um-Gm-Cm- Uf-Am-Uf-Gf-Cf-Cm-Um- Cm-Am-Um-Cf-Um-Um-Cm- Um	112	7	AmsEVP-Gfs-Am-Af-Gf-Af-Um- Gm-Am-Gm-Gm-Cm-Am-Uf-Am- Gf-Cm-Am-Gm-Cm-Ams-Gms-Gm	219	14
A416	B41 6- DV2 8P	Ums-Gms-Cm-Um-Gm-Cm- Uf-Am-Uf-Gm-Cf-Cm-Um- Cm-Am-Um-Cf-Um-Um-Cm- Um	111	7	AmsEVP-Gfs-Af-Af-Gm-Af-Um- Gm-Am-Gm-Gm-Cm-Am-Uf-Am- Gf-Cm-Am-Gm-Cm-Ams-Gms-Gm	220	14
A416	B41 6- DV3 2P	Ums-Gms-Cm-Um-Gm-Cm- Uf-Am-Uf-Gm-Cf-Cm-Um- Cm-Am-Um-Cf-Um-Um-Cm- Um	111	7	AmsEVP-Gfs-Am-Am-Gf-Af-Um- Gm-Am-Gm-Gm-Cm-Am-Uf-Am- Gf-Cm-Am-Gm-Cm-Ams-Gms-Gm	221	14
A416	B41 6- DV3 4P	Ums-Gms-Cm-Um-Gm-Cm- Uf-Am-Uf-Gm-Cf-Cm-Um- Cm-Am-Um-Cf-Um-Um-Cm- Um	111	7	AmsEVP-Gfs-Af-Am-Gm-Af-Um- Gm-Am-Gm-Gm-Cm-Am-Uf-Am- Gf-Cm-Am-Gm-Cm-Ams-Gms-Gm	222	14
A416	B41 6- DV3 0P	Ums-Gms-Cm-Um-Gm-Cm- Uf-Am-Uf-Gf-Cf-Cm-Um- Cm-Am-Um-Cm-Um-Um- Cm-Um	113	7	AmsEVP-Gfs-Am-Am-Gm-Af-Um- Gm-Am-Gm-Gm-Cm-Am-Uf-Am- Gm-Cm-Am-Gm-Cm-Ams-Gms- Gm	223	14
A416	B41 6- DV3 1P	Ums-Gms-Cm-Um-Gm-Cm- Uf-Am-Uf-Gf-Cm-Cm-Um- Cm-Am-Um-Cm-Um-Um- Cm-Um	114	7	AmsEVP-Gfs-Am-Am-Gm-Af-Um- Gm-Am-Gm-Gm-Cm-Am-Uf-Am- Gm-Cf-Am-Gm-Cm-Ams-Gms-Gm	224	14
A416	B41 6- DV2 1P	Ums-Gms-Cm-Um-Gm-Cm- Uf-Am-Uf-Gf-Cf-Cm-Um- Cm-Am-Uf-Cf-Um-Um-Cm- Um	115	7	AmsEVP-Gfs-Am-Am-Gm-Af-Um- Gm-Am-Gm-Gm-Cm-Am-Uf-Am- Gm-Cf-Am-Gm-Cm-Ams-Gms-Gm	224	14

A416	B41 6- DV2 2P	Ums-Gms-Cm-Um-Gm-Cm- Uf-Am-Uf-Gf-Cf-Cm-Um- Cm-Am-Um-Cm-Um-Um- Cm-Um	113	7	AmsEVP-Gfs-Am-Am-Gm-Af-Um- Gm-Am-Gm-Gm-Cm-Am-Uf-Am- Gm-Cf-Am-Gm-Cm-Ams-Gms-Gm	224	14
A205	A20 5- DV2 9P	Gms-Cms-Gm-Gm-Gm-Gm- Uf-Um-Uf-Um-Uf-Cm-Um- Um-Gm-Um-Uf-Gm-Am-Cm- Am	116	18	UmsEVP-Gfs-Um-Cf-Af-Af-Cm- Am-Am-Gm-Am-Am-Am-Af-Am- Cf-Cm-Cm-Cm-Gm-Cms-Cms-Um	225	50
A205	B20 5- DV2 6P	Gms-Cms-Gm-Gm-Gm-Gm- Uf-Um-Uf-Uf-Uf-Cm-Um- Um-Gm-Um-Uf-Gm-Am-Cm- Am	117	18	UmsEVP-Gfs-Uf-Cf-Am-Af-Cm- Am-Am-Gm-Am-Am-Am-Af-Am- Cf-Cm-Cm-Cm-Gm-Cms-Cms-Um	226	50
A205	B20 5- DV2 7P	Gms-Cms-Gm-Gm-Gm-Gm- Uf-Um-Uf-Uf-Uf-Cm-Um- Um-Gm-Um-Uf-Gm-Am-Cm- Am	117	18	UmsEVP-Gfs-Um-Cf-Af-Af-Cm- Am-Am-Gm-Am-Am-Am-Af-Am- Cf-Cm-Cm-Cm-Gm-Cms-Cms-Um	225	50
A205	B20 5- DV2 8P	Gms-Cms-Gm-Gm-Gm-Gm- Uf-Um-Uf-Um-Uf-Cm-Um- Um-Gm-Um-Uf-Gm-Am-Cm- Am	116	18	UmsEVP-Gfs-Uf-Cf-Am-Af-Cm- Am-Am-Gm-Am-Am-Am-Af-Am- Cf-Cm-Cm-Cm-Gm-Cms-Cms-Um	226	50
A205	B20 5- DV3 2P	Gms-Cms-Gm-Gm-Gm-Gm- Uf-Um-Uf-Um-Uf-Cm-Um- Um-Gm-Um-Uf-Gm-Am-Cm- Am	116	18	UmsEVP-Gfs-Um-Cm-Af-Af-Cm- Am-Am-Gm-Am-Am-Am-Af-Am- Cf-Cm-Cm-Cm-Gm-Cms-Cms-Um	227	50
A205	B20 5- DV3 4P	Gms-Cms-Gm-Gm-Gm-Gm- Uf-Um-Uf-Um-Uf-Cm-Um- Um-Gm-Um-Uf-Gm-Am-Cm- Am	116	18	UmsEVP-Gfs-Uf-Cm-Am-Af-Cm- Am-Am-Gm-Am-Am-Am-Af-Am- Cf-Cm-Cm-Cm-Gm-Cms-Cms-Um	228	50
A205	B20 5- DV3 0P	Gms-Cms-Gm-Gm-Gm-Gm- Uf-Um-Uf-Uf-Uf-Cm-Um- Um-Gm-Um-Um-Gm-Am- Cm-Am	118	18	UmsEVP-Gfs-Um-Cm-Am-Af-Cm- Am-Am-Gm-Am-Am-Am-Af-Am- Cm-Cm-Cm-Cm-Gm-Cms-Cms- Um	229	50
A205	B20 5- DV3 1P	Gms-Cms-Gm-Gm-Gm-Gm- Uf-Um-Uf-Uf-Um-Cm-Um- Um-Gm-Um-Um-Gm-Am- Cm-Am	119	18	UmsEVP-Gfs-Um-Cm-Am-Af-Cm- Am-Am-Gm-Am-Am-Am-Af-Am- Cf-Cm-Cm-Cm-Gm-Cms-Cms-Um	230	50
A205	B20 5- DV2 1P	Gms-Cms-Gm-Gm-Gm-Gm- Uf-Um-Uf-Uf-Uf-Cm-Um- Um-Gm-Uf-Uf-Gm-Am-Cm- Am	120	18	UmsEVP-Gfs-Um-Cm-Am-Af-Cm- Am-Am-Gm-Am-Am-Am-Af-Am- Cf-Cm-Cm-Cm-Gm-Cms-Cms-Um	230	50
A205	B20 5- DV2 2P	Gms-Cms-Gm-Gm-Gm-Gm- Uf-Um-Uf-Uf-Uf-Cm-Um- Um-Gm-Um-Um-Gm-Am- Cm-Am	118	18	UmsEVP-Gfs-Um-Cm-Am-Af-Cm- Am-Am-Gm-Am-Am-Am-Af-Am- Cf-Cm-Cm-Cm-Gm-Cms-Cms-Um	230	50

A261	A26 1- DV2 9P	Gms-Ums-Gm-Gm-Am-Cm- Uf-Um-Cf-Um-Cf-Um-Cm- Am-Am-Um-Uf-Um-Um-Cm- Um	126	25	AmsEVP-Gfs-Am-Af-Af-Af-Um- Um-Gm-Am-Gm-Am-Gm-Af-Am- Gf-Um-Cm-Cm-Am-Cms-Cms-Am	237	57
A261	B26 1- DV2 6P	Gms-Ums-Gm-Gm-Am-Cm- Uf-Um-Cf-Uf-Cf-Um-Cm- Am-Am-Um-Uf-Um-Um-Cm- Um	127	25	AmsEVP-Gfs-Af-Af-Am-Af-Um- Um-Gm-Am-Gm-Am-Gm-Af-Am- Gf-Um-Cm-Cm-Am-Cms-Cms-Am	238	57
A261	B26 1- DV2 7P	Gms-Ums-Gm-Gm-Am-Cm- Uf-Um-Cf-Uf-Cf-Um-Cm- Am-Am-Um-Uf-Um-Um-Cm- Um	127	25	AmsEVP-Gfs-Am-Af-Af-Af-Um- Um-Gm-Am-Gm-Am-Gm-Af-Am- Gf-Um-Cm-Cm-Am-Cms-Cms-Am	237	57
A261	B26 1- DV2 8P	Gms-Ums-Gm-Gm-Am-Cm- Uf-Um-Cf-Um-Cf-Um-Cm- Am-Am-Um-Uf-Um-Um-Cm- Um	126	25	AmsEVP-Gfs-Af-Af-Am-Af-Um- Um-Gm-Am-Gm-Am-Gm-Af-Am- Gf-Um-Cm-Cm-Am-Cms-Cms-Am	238	57
A261	B26 1- DV3 2P	Gms-Ums-Gm-Gm-Am-Cm- Uf-Um-Cf-Um-Cf-Um-Cm- Am-Am-Um-Uf-Um-Um-Cm- Um	126	25	AmsEVP-Gfs-Am-Am-Af-Af-Um- Um-Gm-Am-Gm-Am-Gm-Af-Am- Gf-Um-Cm-Cm-Am-Cms-Cms-Am	239	57
A261	B26 1- DV3 4P	Gms-Ums-Gm-Gm-Am-Cm- Uf-Um-Cf-Um-Cf-Um-Cm- Am-Am-Um-Uf-Um-Um-Cm- Um	126	25	AmsEVP-Gfs-Af-Am-Am-Af-Um- Um-Gm-Am-Gm-Am-Gm-Af-Am- Gf-Um-Cm-Cm-Am-Cms-Cms-Am	240	57
A261	B26 1- DV3 0P	Gms-Ums-Gm-Gm-Am-Cm- Uf-Um-Cf-Uf-Cf-Um-Cm- Am-Am-Um-Um-Um-Um- Cm-Um	128	25	AmsEVP-Gfs-Am-Am-Am-Af-Um- Um-Gm-Am-Gm-Am-Gm-Af-Am- Gm-Um-Cm-Cm-Am-Cms-Cms- Am	241	57
A261	B26 1- DV3 1P	Gms-Ums-Gm-Gm-Am-Cm- Uf-Um-Cf-Uf-Cm-Um-Cm- Am-Am-Um-Um-Um-Um- Cm-Um	129	25	AmsEVP-Gfs-Am-Am-Am-Af-Um- Um-Gm-Am-Gm-Am-Gm-Af-Am- Gf-Um-Cm-Cm-Am-Cms-Cms-Am	242	57
A261	B26 1- DV2 1P	Gms-Ums-Gm-Gm-Am-Cm- Uf-Um-Cf-Uf-Cf-Um-Cm- Am-Am-Uf-Uf-Um-Um-Cm- Um	130	25	AmsEVP-Gfs-Am-Am-Am-Af-Um- Um-Gm-Am-Gm-Am-Gm-Af-Am- Gf-Um-Cm-Cm-Am-Cms-Cms-Am	242	57
A261	B26 1- DV2 2P	Gms-Ums-Gm-Gm-Am-Cm- Uf-Um-Cf-Uf-Cf-Um-Cm- Am-Am-Um-Um-Um-Um- Cm-Um	128	25	AmsEVP-Gfs-Am-Am-Am-Af-Um- Um-Gm-Am-Gm-Am-Gm-Af-Am- Gf-Um-Cm-Cm-Am-Cms-Cms-Am	242	57
B208	B20 8- DV2 9P	Gms-Gms-Gm-Um-Um-Um- Uf-Um-Cf-Um-Uf-Gm-Um- Um-Gm-Am-Cf-Am-Am-Gm- Am	121	38	UmsEVP-Cfs-Um-Uf-Gf-Uf-Cm- Am-Am-Cm-Am-Am-Gm-Af-Am- Af-Am-Am-Cm-Cm-Cms-Cms-Gm	231	70

B208	B20 8- DV2 6P	Gms-Gms-Gm-Um-Um-Um- Uf-Um-Cf-Uf-Uf-Gm-Um- Um-Gm-Am-Cf-Am-Am-Gm- Am	122	38	UmsEVP-Cfs-Uf-Uf-Gm-Uf-Cm- Am-Am-Cm-Am-Am-Gm-Af-Am- Af-Am-Am-Cm-Cm-Cms-Cms-Gm	232	70
B208	B20 8- DV2 7P	Gms-Gms-Gm-Um-Um-Um- Uf-Um-Cf-Uf-Uf-Gm-Um- Um-Gm-Am-Cf-Am-Am-Gm- Am	122	38	UmsEVP-Cfs-Um-Uf-Gf-Uf-Cm- Am-Am-Cm-Am-Am-Gm-Af-Am- Af-Am-Am-Cm-Cm-Cms-Cms-Gm	231	70
B208	B20 8- DV2 8P	Gms-Gms-Gm-Um-Um-Um- Uf-Um-Cf-Um-Uf-Gm-Um- Um-Gm-Am-Cf-Am-Am-Gm- Am	121	38	UmsEVP-Cfs-Uf-Uf-Gm-Uf-Cm- Am-Am-Cm-Am-Am-Gm-Af-Am- Af-Am-Am-Cm-Cm-Cms-Cms-Gm	232	70
B208	B20 8- DV3 2P	Gms-Gms-Gm-Um-Um-Um- Uf-Um-Cf-Um-Uf-Gm-Um- Um-Gm-Am-Cf-Am-Am-Gm- Am	121	38	UmsEVP-Cfs-Um-Um-Gf-Uf-Cm- Am-Am-Cm-Am-Am-Gm-Af-Am- Af-Am-Am-Cm-Cm-Cms-Cms-Gm	233	70
B208	B20 8- DV3 4P	Gms-Gms-Gm-Um-Um-Um- Uf-Um-Cf-Um-Uf-Gm-Um- Um-Gm-Am-Cf-Am-Am-Gm- Am	121	38	UmsEVP-Cfs-Uf-Um-Gm-Uf-Cm- Am-Am-Cm-Am-Am-Gm-Af-Am- Af-Am-Am-Cm-Cm-Cms-Cms-Gm	234	70
B208	B20 8- DV3 0P	Gms-Gms-Gm-Um-Um-Um- Uf-Um-Cf-Uf-Uf-Gm-Um- Um-Gm-Am-Cm-Am-Am- Gm-Am	123	38	UmsEVP-Cfs-Um-Um-Gm-Uf-Cm- Am-Am-Cm-Am-Am-Gm-Af-Am- Am-Am-Am-Cm-Cm-Cms-Cms- Gm	235	70
B208	B20 8- DV3 1P	Gms-Gms-Gm-Um-Um-Um- Uf-Um-Cf-Uf-Um-Gm-Um- Um-Gm-Am-Cm-Am-Am- Gm-Am	124	38	UmsEVP-Cfs-Um-Um-Gm-Uf-Cm- Am-Am-Cm-Am-Am-Gm-Af-Am- Af-Am-Am-Cm-Cm-Cms-Cms-Gm	236	70
B208	B20 8- DV2 1P	Gms-Gms-Gm-Um-Um-Um- Uf-Um-Cf-Uf-Uf-Gm-Um- Um-Gm-Af-Cf-Am-Am-Gm- Am	125	38	UmsEVP-Cfs-Um-Um-Gm-Uf-Cm- Am-Am-Cm-Am-Am-Gm-Af-Am- Af-Am-Am-Cm-Cm-Cms-Cms-Gm	236	70
B208	B20 8- DV2 2P	Gms-Gms-Gm-Um-Um-Um- Uf-Um-Cf-Uf-Uf-Gm-Um- Um-Gm-Am-Cm-Am-Am- Gm-Am	123	38	UmsEVP-Cfs-Um-Um-Gm-Uf-Cm- Am-Am-Cm-Am-Am-Gm-Af-Am- Af-Am-Am-Cm-Cm-Cms-Cms-Gm	236	70
B262	B26 2- DV2 9P	Ums-Gms-Gm-Am-Cm-Um- Uf-Cm-Uf-Cm-Uf-Cm-Am- Am-Um-Um-Uf-Um-Cm-Um- Am	131	39	UmsEVP-Afs-Gm-Af-Af-Af-Am- Um-Um-Gm-Am-Gm-Am-Gf-Am- Af-Gm-Um-Cm-Cm-Ams-Cms-Gm	243	71
B262	B26 2- DV2 6P	Ums-Gms-Gm-Am-Cm-Um- Uf-Cm-Uf-Cf-Uf-Cm-Am- Am-Um-Um-Uf-Um-Cm-Um- Am	132	39	UmsEVP-Afs-Gf-Af-Am-Af-Am- Um-Um-Gm-Am-Gm-Am-Gf-Am- Af-Gm-Um-Cm-Cm-Ams-Cms-Gm	244	71

B262	B26 2- DV2 7P	Ums-Gms-Gm-Am-Cm-Um- Uf-Cm-Uf-Cf-Uf-Cm-Am- Am-Um-Um-Uf-Um-Cm-Um- Am	132	39	UmsEVP-Afs-Gm-Af-Af-Af-Am- Um-Um-Gm-Am-Gm-Am-Gf-Am- Af-Gm-Um-Cm-Cm-Ams-Cms-Gm	243	71
B262	B26 2- DV2 8P	Ums-Gms-Gm-Am-Cm-Um- Uf-Cm-Uf-Cm-Uf-Cm-Am- Am-Um-Um-Uf-Um-Cm-Um- Am	131	39	UmsEVP-Afs-Gf-Af-Am-Af-Am- Um-Um-Gm-Am-Gm-Am-Gf-Am- Af-Gm-Um-Cm-Cm-Ams-Cms-Gm	244	71
B262	B26 2- DV3 2P	Ums-Gms-Gm-Am-Cm-Um- Uf-Cm-Uf-Cm-Uf-Cm-Am- Am-Um-Um-Uf-Um-Cm-Um- Am	131	39	UmsEVP-Afs-Gm-Am-Af-Af-Am- Um-Um-Gm-Am-Gm-Am-Gf-Am- Af-Gm-Um-Cm-Cm-Ams-Cms-Gm	245	71
B262	B26 2- DV3 4P	Ums-Gms-Gm-Am-Cm-Um- Uf-Cm-Uf-Cm-Uf-Cm-Am- Am-Um-Um-Uf-Um-Cm-Um- Am	131	39	UmsEVP-Afs-Gf-Am-Am-Af-Am- Um-Um-Gm-Am-Gm-Am-Gf-Am- Af-Gm-Um-Cm-Cm-Ams-Cms-Gm	246	71
B262	B26 2- DV3 0P	Ums-Gms-Gm-Am-Cm-Um- Uf-Cm-Uf-Cf-Uf-Cm-Am- Am-Um-Um-Um-Um-Cm- Um-Am	133	39	UmsEVP-Afs-Gm-Am-Am-Af-Am- Um-Um-Gm-Am-Gm-Am-Gf-Am- Am-Gm-Um-Cm-Cm-Ams-Cms- Gm	247	71
B262	B26 2- DV3 1P	Ums-Gms-Gm-Am-Cm-Um- Uf-Cm-Uf-Cf-Um-Cm-Am- Am-Um-Um-Um-Um-Cm- Um-Am	134	39	UmsEVP-Afs-Gm-Am-Am-Af-Am- Um-Um-Gm-Am-Gm-Am-Gf-Am- Af-Gm-Um-Cm-Cm-Ams-Cms-Gm	248	71
B262	B26 2- DV2 1P	Ums-Gms-Gm-Am-Cm-Um- Uf-Cm-Uf-Cf-Uf-Cm-Am- Am-Um-Uf-Uf-Um-Cm-Um- Am	135	39	UmsEVP-Afs-Gm-Am-Am-Af-Am- Um-Um-Gm-Am-Gm-Am-Gf-Am- Af-Gm-Um-Cm-Cm-Ams-Cms-Gm	248	71
B262	B26 2- DV2 2P	Ums-Gms-Gm-Am-Cm-Um- Uf-Cm-Uf-Cf-Uf-Cm-Am- Am-Um-Um-Um-Um-Cm- Um-Am	133	39	UmsEVP-Afs-Gm-Am-Am-Af-Am- Um-Um-Gm-Am-Gm-Am-Gf-Am- Af-Gm-Um-Cm-Cm-Ams-Cms-Gm	248	71
B264	B26 4- DV2 9P	Gms-Ams-Cm-Um-Um-Cm- Uf-Cm-Uf-Cm-Af-Am-Um- Um-Um-Um-Cf-Um-Am-Gm- Am	136	40	UmsEVP-Cfs-Um-Af-Gf-Af-Am- Am-Am-Um-Um-Gm-Am-Gf-Am- Gf-Am-Am-Gm-Um-Cms-Cms-Am	249	72
B264	B26 4- DV2 6P	Gms-Ams-Cm-Um-Um-Cm- Uf-Cm-Uf-Cf-Af-Am-Um- Um-Um-Um-Cf-Um-Am-Gm- Am	137	40	UmsEVP-Cfs-Uf-Af-Gm-Af-Am- Am-Am-Um-Um-Gm-Am-Gf-Am- Gf-Am-Am-Gm-Um-Cms-Cms-Am	250	72
B264	B26 4- DV2 7P	Gms-Ams-Cm-Um-Um-Cm- Uf-Cm-Uf-Cf-Af-Am-Um- Um-Um-Um-Cf-Um-Am-Gm- Am	137	40	UmsEVP-Cfs-Um-Af-Gf-Af-Am- Am-Am-Um-Um-Gm-Am-Gf-Am- Gf-Am-Am-Gm-Um-Cms-Cms-Am	249	72

B264	B26 4- DV2 8P	Gms-Ams-Cm-Um-Um-Cm- Uf-Cm-Uf-Cm-Af-Am-Um- Um-Um-Um-Cf-Um-Am-Gm- Am	136	40	UmsEVP-Cfs-Uf-Af-Gm-Af-Am- Am-Am-Um-Um-Gm-Am-Gf-Am- Gf-Am-Am-Gm-Um-Cms-Cms-Am	250	72
B264	B26 4- DV3 2P	Gms-Ams-Cm-Um-Um-Cm- Uf-Cm-Uf-Cm-Af-Am-Um- Um-Um-Um-Cf-Um-Am-Gm- Am	136	40	UmsEVP-Cfs-Um-Am-Gf-Af-Am- Am-Am-Um-Um-Gm-Am-Gf-Am- Gf-Am-Am-Gm-Um-Cms-Cms-Am	251	72
B264	B26 4- DV3 4P	Gms-Ams-Cm-Um-Um-Cm- Uf-Cm-Uf-Cm-Af-Am-Um- Um-Um-Um-Cf-Um-Am-Gm- Am	136	40	UmsEVP-Cfs-Uf-Am-Gm-Af-Am- Am-Am-Um-Um-Gm-Am-Gf-Am- Gf-Am-Am-Gm-Um-Cms-Cms-Am	252	72
B264	B26 4- DV3 0P	Gms-Ams-Cm-Um-Um-Cm- Uf-Cm-Uf-Cf-Af-Am-Um- Um-Um-Um-Cm-Um-Am- Gm-Am	138	40	UmsEVP-Cfs-Um-Am-Gm-Af-Am- Am-Am-Um-Um-Gm-Am-Gf-Am- Gm-Am-Am-Gm-Um-Cms-Cms- Am	253	72
B264	B26 4- DV3 1P	Gms-Ams-Cm-Um-Um-Cm- Uf-Cm-Uf-Cf-Am-Am-Um- Um-Um-Um-Cm-Um-Am- Gm-Am	139	40	UmsEVP-Cfs-Um-Am-Gm-Af-Am- Am-Am-Um-Um-Gm-Am-Gf-Am- Gf-Am-Am-Gm-Um-Cms-Cms-Am	254	72
B264	B26 4- DV2 1P	Gms-Ams-Cm-Um-Um-Cm- Uf-Cm-Uf-Cf-Af-Am-Um- Um-Um-Uf-Cf-Um-Am-Gm- Am	140	40	UmsEVP-Cfs-Um-Am-Gm-Af-Am- Am-Am-Um-Um-Gm-Am-Gf-Am- Gf-Am-Am-Gm-Um-Cms-Cms-Am	254	72
B264	B26 4- DV2 2P	Gms-Ams-Cm-Um-Um-Cm- Uf-Cm-Uf-Cf-Af-Am-Um- Um-Um-Um-Cm-Um-Am- Gm-Am	138	40	UmsEVP-Cfs-Um-Am-Gm-Af-Am- Am-Am-Um-Um-Gm-Am-Gf-Am- Gf-Am-Am-Gm-Um-Cms-Cms-Am	254	72
B1556	B15 56- DV2 9P	Ums-Gms-Um-Gm-Cm-Cm- Uf-Um-Cf-Um-Cf-Am-Um- Cm-Um-Gm-Cf-Cm-Gm-Gm- Am	141	41	UmsEVP-Cfs-Cm-Gf-Gf-Cf-Am- Gm-Am-Um-Gm-Am-Gm-Af-Am- Gf-Gm-Cm-Am-Cm-Ams-Gms-Am	255	73
B1556	B15 56- DV2 6P	Ums-Gms-Um-Gm-Cm-Cm- Uf-Um-Cf-Uf-Cf-Am-Um- Cm-Um-Gm-Cf-Cm-Gm-Gm- Am	142	41	UmsEVP-Cfs-Cf-Gf-Gm-Cf-Am- Gm-Am-Um-Gm-Am-Gm-Af-Am- Gf-Gm-Cm-Am-Cm-Ams-Gms-Am	256	73
B1556	B15 56- DV2 7P	Ums-Gms-Um-Gm-Cm-Cm- Uf-Um-Cf-Uf-Cf-Am-Um- Cm-Um-Gm-Cf-Cm-Gm-Gm- Am	142	41	UmsEVP-Cfs-Cm-Gf-Gf-Cf-Am- Gm-Am-Um-Gm-Am-Gm-Af-Am- Gf-Gm-Cm-Am-Cm-Ams-Gms-Am	255	73
B1556	B15 56- DV2 8P	Ums-Gms-Um-Gm-Cm-Cm- Uf-Um-Cf-Um-Cf-Am-Um- Cm-Um-Gm-Cf-Cm-Gm-Gm- Am	141	41	UmsEVP-Cfs-Cf-Gf-Gm-Cf-Am- Gm-Am-Um-Gm-Am-Gm-Af-Am- Gf-Gm-Cm-Am-Cm-Ams-Gms-Am	256	73

B1556	B15 56- DV3 2P	Ums-Gms-Um-Gm-Cm-Cm- Uf-Um-Cf-Um-Cf-Am-Um- Cm-Um-Gm-Cf-Cm-Gm-Gm- Am	141	41	UmsEVP-Cfs-Cm-Gm-Gf-Cf-Am- Gm-Am-Um-Gm-Am-Gm-Af-Am- Gf-Gm-Cm-Am-Cm-Ams-Gms-Am	257	73
B1556	B15 56- DV3 4P	Ums-Gms-Um-Gm-Cm-Cm- Uf-Um-Cf-Um-Cf-Am-Um- Cm-Um-Gm-Cf-Cm-Gm-Gm- Am	141	41	UmsEVP-Cfs-Cf-Gm-Gm-Cf-Am- Gm-Am-Um-Gm-Am-Gm-Af-Am- Gf-Gm-Cm-Am-Cm-Ams-Gms-Am	258	73
B1556	B15 56- DV3 0P	Ums-Gms-Um-Gm-Cm-Cm- Uf-Um-Cf-Uf-Cf-Am-Um- Cm-Um-Gm-Cm-Cm-Gm- Gm-Am	143	41	UmsEVP-Cfs-Cm-Gm-Gm-Cf-Am- Gm-Am-Um-Gm-Am-Gm-Af-Am- Gm-Gm-Cm-Am-Cm-Ams-Gms- Am	259	73
B1556	B15 56- DV3 1P	Ums-Gms-Um-Gm-Cm-Cm- Uf-Um-Cf-Uf-Cm-Am-Um- Cm-Um-Gm-Cm-Cm-Gm- Gm-Am	144	41	UmsEVP-Cfs-Cm-Gm-Gm-Cf-Am- Gm-Am-Um-Gm-Am-Gm-Af-Am- Gf-Gm-Cm-Am-Cm-Ams-Gms-Am	260	73
B1556	B15 56- DV2 1P	Ums-Gms-Um-Gm-Cm-Cm- Uf-Um-Cf-Uf-Cf-Am-Um- Cm-Um-Gf-Cf-Cm-Gm-Gm- Am	145	41	UmsEVP-Cfs-Cm-Gm-Gm-Cf-Am- Gm-Am-Um-Gm-Am-Gm-Af-Am- Gf-Gm-Cm-Am-Cm-Ams-Gms-Am	260	73
B1556	B15 56- DV2 2P	Ums-Gms-Um-Gm-Cm-Cm- Uf-Um-Cf-Uf-Cf-Am-Um- Cm-Um-Gm-Cm-Cm-Gm- Gm-Am	143	41	UmsEVP-Cfs-Cm-Gm-Gm-Cf-Am- Gm-Am-Um-Gm-Am-Gm-Af-Am- Gf-Gm-Cm-Am-Cm-Ams-Gms-Am	260	73
B1560	B15 60- DV2 9P	Cms-Cms-Um-Um-Cm-Um- Cf-Am-Uf-Cm-Uf-Gm-Cm- Cm-Gm-Gm-Af-Cm-Cm-Gm- Um	146	42	AmsEVP-Cfs-Gm-Gf-Uf-Cf-Cm- Gm-Gm-Cm-Am-Gm-Am-Uf-Gm- Af-Gm-Am-Am-Gm-Gms-Cms-Am	261	74
B1560	B15 60- DV2 6P	Cms-Cms-Um-Um-Cm-Um- Cf-Am-Uf-Cf-Uf-Gm-Cm- Cm-Gm-Gm-Af-Cm-Cm-Gm- Um	147	42	AmsEVP-Cfs-Gf-Gf-Um-Cf-Cm- Gm-Gm-Cm-Am-Gm-Am-Uf-Gm- Af-Gm-Am-Am-Gm-Gms-Cms-Am	262	74
B1560	B15 60- DV2 7P	Cms-Cms-Um-Um-Cm-Um- Cf-Am-Uf-Cf-Uf-Gm-Cm- Cm-Gm-Gm-Af-Cm-Cm-Gm- Um	147	42	AmsEVP-Cfs-Gm-Gf-Uf-Cf-Cm- Gm-Gm-Cm-Am-Gm-Am-Uf-Gm- Af-Gm-Am-Am-Gm-Gms-Cms-Am	261	74
B1560	B15 60- DV2 8P	Cms-Cms-Um-Um-Cm-Um- Cf-Am-Uf-Cm-Uf-Gm-Cm- Cm-Gm-Gm-Af-Cm-Cm-Gm- Um	146	42	AmsEVP-Cfs-Gf-Gf-Um-Cf-Cm- Gm-Gm-Cm-Am-Gm-Am-Uf-Gm- Af-Gm-Am-Am-Gm-Gms-Cms-Am	262	74
B1560	B15 60- DV3 2P	Cms-Cms-Um-Um-Cm-Um- Cf-Am-Uf-Cm-Uf-Gm-Cm- Cm-Gm-Gm-Af-Cm-Cm-Gm- Um	146	42	AmsEVP-Cfs-Gm-Gm-Uf-Cf-Cm- Gm-Gm-Cm-Am-Gm-Am-Uf-Gm- Af-Gm-Am-Am-Gm-Gms-Cms-Am	263	74

B1560	B15 60- DV3 4P	Cms-Cms-Um-Um-Cm-Um- Cf-Am-Uf-Cm-Uf-Gm-Cm- Cm-Gm-Gm-Af-Cm-Cm-Gm- Um	146	42	AmsEVP-Cfs-Gf-Gm-Um-Cf-Cm- Gm-Gm-Cm-Am-Gm-Am-Uf-Gm- Af-Gm-Am-Am-Gm-Gms-Cms-Am	264	74
B1560	B15 60- DV3 0P	Cms-Cms-Um-Um-Cm-Um- Cf-Am-Uf-Cf-Uf-Gm-Cm- Cm-Gm-Gm-Am-Cm-Cm- Gm-Um	148	42	AmsEVP-Cfs-Gm-Gm-Um-Cf-Cm- Gm-Gm-Cm-Am-Gm-Am-Uf-Gm- Am-Gm-Am-Am-Gm-Gms-Cms- Am	265	74
B1560	B15 60- DV3 1P	Cms-Cms-Um-Um-Cm-Um- Cf-Am-Uf-Cf-Um-Gm-Cm- Cm-Gm-Gm-Am-Cm-Cm- Gm-Um	149	42	AmsEVP-Cfs-Gm-Gm-Um-Cf-Cm- Gm-Gm-Cm-Am-Gm-Am-Uf-Gm- Af-Gm-Am-Am-Gm-Gms-Cms-Am	266	74
B1560	B15 60- DV2 1P	Cms-Cms-Um-Um-Cm-Um- Cf-Am-Uf-Cf-Uf-Gm-Cm- Cm-Gm-Gf-Af-Cm-Cm-Gm- Um	150	42	AmsEVP-Cfs-Gm-Gm-Um-Cf-Cm- Gm-Gm-Cm-Am-Gm-Am-Uf-Gm- Af-Gm-Am-Am-Gm-Gms-Cms-Am	266	74
B1560	B15 60- DV2 2P	Cms-Cms-Um-Um-Cm-Um- Cf-Am-Uf-Cf-Uf-Gm-Cm- Cm-Gm-Gm-Am-Cm-Cm- Gm-Um	148	42	AmsEVP-Cfs-Gm-Gm-Um-Cf-Cm- Gm-Gm-Cm-Am-Gm-Am-Uf-Gm- Af-Gm-Am-Am-Gm-Gms-Cms-Am	266	74
B416S	B41 6S- DV2 9P	Ums-Gms-Cm-Um-Gm-Cf- Um-Af-Um-Gf-Cm-Cm-Um- Cm-Am-Uf-Cm-Um-Um-Am	151	43	UmsEVP-Afs-Am-Gf-Af-Uf-Gm- Am-Gm-Gm-Cm-Am-Um-Af-Gm- Cf-Am-Gm-Cm-Ams-Gms-Gm	267	75
B416S	B41 6S- DV2 6P	Ums-Gms-Cm-Um-Gm-Cf- Um-Af-Uf-Gf-Cm-Cm-Um- Cm-Am-Uf-Cm-Um-Um-Am	152	43	UmsEVP-Afs-Af-Gf-Am-Uf-Gm- Am-Gm-Gm-Cm-Am-Um-Af-Gm- Cf-Am-Gm-Cm-Ams-Gms-Gm	268	75
B416S	B41 6S- DV2 7P	Ums-Gms-Cm-Um-Gm-Cf- Um-Af-Uf-Gf-Cm-Cm-Um- Cm-Am-Uf-Cm-Um-Um-Am	152	43	UmsEVP-Afs-Am-Gf-Af-Uf-Gm- Am-Gm-Gm-Cm-Am-Um-Af-Gm- Cf-Am-Gm-Cm-Ams-Gms-Gm	267	75
B416S	B41 6S- DV2 8P	Ums-Gms-Cm-Um-Gm-Cf- Um-Af-Um-Gf-Cm-Cm-Um- Cm-Am-Uf-Cm-Um-Um-Am	151	43	UmsEVP-Afs-Af-Gf-Am-Uf-Gm- Am-Gm-Gm-Cm-Am-Um-Af-Gm- Cf-Am-Gm-Cm-Ams-Gms-Gm	268	75
B416S	B41 6S- DV3 2P	Ums-Gms-Cm-Um-Gm-Cf- Um-Af-Um-Gf-Cm-Cm-Um- Cm-Am-Uf-Cm-Um-Um-Am	151	43	UmsEVP-Afs-Am-Gm-Af-Uf-Gm- Am-Gm-Gm-Cm-Am-Um-Af-Gm- Cf-Am-Gm-Cm-Ams-Gms-Gm	269	75
B416S	B41 6S- DV3 4P	Ums-Gms-Cm-Um-Gm-Cf- Um-Af-Um-Gf-Cm-Cm-Um- Cm-Am-Uf-Cm-Um-Um-Am	151	43	UmsEVP-Afs-Af-Gm-Am-Uf-Gm- Am-Gm-Gm-Cm-Am-Um-Af-Gm- Cf-Am-Gm-Cm-Ams-Gms-Gm	270	75

B416S	B41 6S- DV3 0P	Ums-Gms-Cm-Um-Gm-Cf- Um-Af-Uf-Gf-Cm-Cm-Um- Cm-Am-Um-Cm-Um-Um-Am	153	43	UmsEVP-Afs-Am-Gm-Am-Uf-Gm- Am-Gm-Gm-Cm-Am-Um-Af-Gm- Cm-Am-Gm-Cm-Ams-Gms-Gm	271	75
B416S	B41 6S- DV3 1P	Ums-Gms-Cm-Um-Gm-Cf- Um-Af-Uf-Gm-Cm-Cm-Um- Cm-Am-Um-Cm-Um-Um-Am	154	43	UmsEVP-Afs-Am-Gm-Am-Uf-Gm- Am-Gm-Gm-Cm-Am-Um-Af-Gm- Cf-Am-Gm-Cm-Ams-Gms-Gm	272	75
B416S	B41 6S- DV2 1P	Ums-Gms-Cm-Um-Gm-Cf- Um-Af-Uf-Gf-Cm-Cm-Um- Cm-Af-Uf-Cm-Um-Um-Am	155	43	UmsEVP-Afs-Am-Gm-Am-Uf-Gm- Am-Gm-Gm-Cm-Am-Um-Af-Gm- Cf-Am-Gm-Cm-Ams-Gms-Gm	272	75
B416S	B41 6S- DV2 2P	Ums-Gms-Cm-Um-Gm-Cf- Um-Af-Uf-Gf-Cm-Cm-Um- Cm-Am-Um-Cm-Um-Um-Am	153	43	UmsEVP-Afs-Am-Gm-Am-Uf-Gm- Am-Gm-Gm-Cm-Am-Um-Af-Gm- Cf-Am-Gm-Cm-Ams-Gms-Gm	272	75

[0413] 2. Experimental methods

[0414] Referring to Example 2, the concentration for the single-concentration screening experiment was set at 0.3 nM.

[0415] 3. Experimental results

[0416] The experimental results demonstrated that the sequences B207S-DV32P, A1550-DV27P, B1572-DV27P, B1575-DV27P, B207S-DV29P, A1573-DV29P, B1575-DV29P, B418S-DV34P, and A416-DV27P exhibited significantly superior inhibitory effects on HBsAg compared to the positive control sequence, as well as significantly superior inhibitory effects on HBeAg compared to the positive control sequence.

[0417] The inhibition rates of each sequence on the expression of HBsAg and HBeAg at a single concentration, as well as their relative inhibition rates compared to the positive control sequence APC-VIR, are shown in Table12-41. The comprehensive ranking represents the inhibition rate ranking of the sequences relative to the positive control.

[0418] The single-concentration screening results indicated that some unmodified sequences modified with the templates DV25P-29P and DV32P-34P of the present disclosure demonstrated superior inhibitory effects on HBsAg and HBeAg compared to the positive control sequence. Among them, the modified sequences designed from the unmodified sequences B207S and B1575 ranked highest in terms of HBsAg inhibition rate, demonstrating

the sensitivity of these target sites. Specifically, the sequences B207S-DV29P, B207S-DV32P, B1575-DV29P, B1575-DV26P, and B1575-DV27P all achieved HBsAg inhibition rates exceeding 90%. For example, the sequences B207S-DV29P and B1575-DV29P achieved HBsAg inhibition rates of 92.11% and 91.78%, respectively. Similarly, the modified sequences designed from the unmodified sequences B1575 and A1573 ranked highest in terms of HBeAg inhibition rate, demonstrating the sensitivity of these target sites. Specifically, the sequences B1575-DV29P, B1575-DV26P, B1575-DV27P, and B1575-DV32P all achieved HBeAg inhibition rates exceeding 60%.

[0419] The activity of the same siRNA sequence can vary significantly when modified with different templates. For example, the unmodified sequence B1556 modified with the template DV27P of the present disclosure showed an increase in HBsAg inhibition rate by 48.63% compared to modification with the template DV28P of the present disclosure, indicating a significant improvement.

[0420] Despite having similar unmodified sequences, the sensitivity to different modification templates varies. For example, the unmodified sequence B1572 modified with the template DV27P of the present disclosure achieved the highest inhibition rates for both HBsAg and HBeAg, while the unmodified sequences A1573 and B1575 achieved the highest inhibition rates for HBsAg and HBeAg when modified with the template DV29P. This demonstrates that it is uncertain which modification template will result in high activity for a given siRNA sequence.

[0421] The IC₅₀ experimental results and cytotoxicity data for each sequence are shown in Table 42. The experimental results showed that the IC₅₀ values of the 11 sequences tested for HBsAg ranged from 0.05 nM to 0.15 nM, all of which were lower than the IC₅₀ value of the positive control sequence (0.3362 nM), demonstrating superior inhibitory effects compared to the positive control. For example, the IC₅₀ values of B1572-DV27P, B1550-DV27P, and B207S-DV32P for HBsAg were 0.0538 nM, 0.0570 nM, and 0.0572 nM, respectively. Except for the sequence B261-DV26P, the IC₅₀ values of the remaining 10 sequences for HBeAg ranged from 0.2 nM to 0.85 nM, all of which were lower than the IC₅₀ value of the positive control sequence (1.89 nM), demonstrating superior inhibitory effects compared to the positive control. For example, the IC₅₀ values of B1550-DV27P, B1572-DV27P, and B1575-DV26P

for HBeAg were 0.2073 nM, 0.2143 nM, and 0.2923 nM, respectively. The cytotoxicity results showed that all 11 sequences exhibited cell viability greater than 90% at the highest tested concentration of 10 nM, indicating no significant cytotoxicity and suggesting a wide concentration range for selection.

[0422] Based on the comprehensive results, the following 10 sequences can be selected as candidate sequences: B207S-DV29P, B207S-DV32P, B1575-DV29P, B1575-DV26P, B1575-DV27P, B1572-DV27P, B1573-DV29P, B1550-DV27P, B416-DV27P, and B418S-DV34P.

[0423] (i) Inhibition of HBsAg and HBeAg by unmodified sequences modified with templates

[0424] (1) unmodified sequence A1550

[0425] Table 12: HBsAg inhibition rates of unmodified sequence A1550 modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
A1550	Modification templates of the present disclosure	A1550-DV29P	83.37	21.62
		B1550-DV26P	87.11	25.36
		B1550-DV27P	87.26	25.51
		B1550-DV28P	75.18	13.43
		B1550-DV32P	80.13	18.38
		B1550-DV33P	82.73	20.98
		B1550-DV34P	83.03	21.28
		B1550-DV25P	83.46	21.71
	Prior art modification templates	B1550-DV30P	68.31	NA
		B1550-DV31P	61.75	NA
		B1550-DV21P	62.24	NA
		B1550-DV22P	68.71	NA

[0426] Table 13: HBeAg inhibition rates of unmodified sequence A1550 modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over prior art templates (%)
A1550	Modification templates of the present disclosure	A1550-DV29P	46.07	20.17
		B1550-DV26P	50.67	24.77
		B1550-DV27P	52.45	26.55
		B1550-DV28P	38.47	12.57
		B1550-DV32P	43.41	17.51
		B1550-DV33P	44.94	19.04
		B1550-DV34P	46.22	20.32
		B1550-DV25P	46.23	20.33
		B1550-DV30P	29.23	NA
		B1550-DV31P	26.28	NA

	Prior art modification templates	B1550-DV21P	25.90	NA
		B1550-DV22P	33.52	NA

[0427] When the unmodified sequence A1550 was modified using the templates DV25P-29P and DV32P-34P of the present disclosure, the inhibition rates for both HBsAg and HBeAg were increased compared to those modified with prior art templates. For example, when modified with templates DV26P and DV27P, the sequences B1550-DV26P and B1550-DV27P showed increases in HBsAg inhibition rates by 25.36% and 25.51%, respectively, and increases in HBeAg inhibition rates by 24.77% and 26.55%, respectively. Detailed results are shown in Table 12 and Table 13.

[0428] (2) unmodified sequence B1556

[0429] Table 14: HBsAg inhibition rates of unmodified sequence B1556 modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B1556	Modification templates of the present disclosure	B1556-DV29P	42.51	13.57
		B1556-DV26P	66.4	37.46
		B1556-DV27P	76.55	47.61
		B1556-DV28P	27.92	-1.02
		B1556-DV32P	31.7	2.76
		B1556-DV34P	32.09	3.15
	Prior art modification templates	B1556-DV30P	29.10	NA
		B1556-DV31P	29.13	NA
		B1556-DV21P	28.94	NA
		B1556-DV22P	34.79	NA

[0430] Table 15: HBeAg inhibition rates of unmodified sequence B1556 modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B1556	Modification templates of the	B1556-DV29P	16.37	4.70
		B1556-DV26P	36.96	25.29
		B1556-DV27P	43.4	31.73

	present disclosure	B1556-DV28P	8.87	-2.80
		B1556-DV32P	8.36	-3.31
		B1556-DV34P	9.71	-1.96
	Prior art modification templates	B1556-DV30P	12.52	NA
		B1556-DV31P	11.67	NA
		B1556-DV21P	12.28	NA
		B1556-DV22P	20.42	NA

[0431] The unmodified sequence B1556 was modified using the templates DV26P-29P, DV32P and DV34P of the present disclosure. Compared with those modified with prior art templates, the sequences B1556-DV26P and B1556-DV27P modified with templates DV26P and DV27P showed increases in HBsAg inhibition rates by 37.46% and 47.61%, respectively, and increases in HBeAg inhibition rates by 25.29% and 31.73%, respectively. Detailed results are shown in Table 14 and Table 15.

[0432] (3) unmodified sequence B1560

[0433] Table 16: HBsAg inhibition rates of unmodified sequence B1560 modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B1560	Modification templates of the present disclosure	B1560-DV29P	48.04	22.43
		B1560-DV26P	61.43	35.82
		B1560-DV27P	69.21	43.60
		B1560-DV28P	30.71	5.10
		B1560-DV32P	39.72	14.11
		B1560-DV34P	33.94	8.33
	Prior art modification templates	B1560-DV30P	25.61	NA
		B1560-DV31P	27.26	NA
		B1560-DV21P	29.53	NA
		B1560-DV22P	30.68	NA

[0434] Table 17: HBeAg inhibition rates of unmodified sequence B1560 modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over prior art templates (%)
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B1560	Modification templates of the present disclosure	B1560-DV29P	18.12	8.94
		B1560-DV26P	30.02	20.84
		B1560-DV27P	37.72	28.54
		B1560-DV28P	12.67	3.49
		B1560-DV32P	15.59	6.41
		B1560-DV34P	14.96	5.78
	Prior art modification templates	B1560-DV30P	9.18	NA
		B1560-DV31P	12.76	NA
		B1560-DV21P	13.06	NA
		B1560-DV22P	14.74	NA

[0435] The unmodified sequence B1560 was modified using the templates DV26P-29P, DV32P and DV34P of the present disclosure. Compared with those modified with prior art templates, the sequences B1560-DV26P and B1560-DV27P modified with templates DV26P and DV27P showed increases in HBsAg inhibition rates by 35.82% and 43.60%, respectively, and increases in HBeAg inhibition rates by 20.84% and 28.54%, respectively. Detailed results are shown in Table 16 and Table 17.

[0436] From this, the following conclusions can be drawn:

[0437] 1) For the unmodified sequences A1550, B1556, and B1560, modification with the templates DV25P-29P and DV32P-34P of the present disclosure resulted in significantly superior inhibition of both HBsAg and HBeAg compared to those modified with prior art templates. When modified with DV26P and DV27P, the inhibition rates for HBsAg and HBeAg were the highest and significantly better than those achieved with prior art templates.

[0438] 2) The activity of the same siRNA sequence can vary significantly when modified with different templates. For example, the unmodified sequence B1556 modified with the template DV27P of the present disclosure showed an increase in HBsAg inhibition rate by 48.63% compared to modification with the template DV28P of the present disclosure, indicating a significant improvement.

[0439] (3) siRNA sequences with similar sequences can exhibit vastly different levels of activity. For example, the DV29P-modified sequence A1550-DV29P showed a 40.86% higher HBsAg inhibition rate compared to A1556-DV29P.

[0440] (4) unmodified sequence B1572

[0441] Table 18: HBsAg inhibition rates of unmodified sequence B1572 modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B1572	Modification templates of the present disclosure	B1572-DV29P	84	22.76
		B1572-DV26P	88.08	26.84
		B1572-DV27P	88.83	27.59
		B1572-DV28P	80.65	19.41
		B1572-DV32P	75.61	14.37
		B1572-DV34P	78.58	17.34
	Prior art modification templates	B1572-DV30P	65.51	NA
		B1572-DV31P	61.24	NA
		B1572-DV21P	66.07	NA
		B1572-DV22P	67.99	NA

[0442] Table 19: HBeAg inhibition rates of unmodified sequence B1572 modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B1572	Modification templates of the present disclosure	B1572-DV29P	48.73	25.68
		B1572-DV26P	55.07	32.02
		B1572-DV27P	59.79	36.74
		B1572-DV28P	46.44	23.39
		B1572-DV32P	40.05	17.00
		B1572-DV34P	43.26	20.21
	Prior art modification templates	B1572-DV30P	34.57	NA
		B1572-DV31P	23.05	NA
		B1572-DV21P	35.98	NA
		B1572-DV22P	40.04	NA

[0443] The unmodified sequence B1572 was modified using the templates DV26P-29P, DV32P and DV34P of the present disclosure. Compared with those modified with prior art templates, the sequences B1572-DV26P and B1572-DV27P modified with templates DV26P and DV27P showed increases in HBsAg inhibition rates by 26.84% and 27.59%, respectively, and increases in HBeAg inhibition rates by 32.02% and 36.74%, respectively. Detailed results are shown in Table 18 and Table 19.

[0444] (5) unmodified sequence A1573

[0445] Table 20: HBsAg inhibition rates of unmodified sequence A1573 modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
A1573	Modification templates of the present disclosure	A1573-DV29P	85.06	16.08
		B1573-DV26P	81.89	12.91
		B1573-DV27P	83.93	14.95
		B1573-DV28P	82.81	13.83
		B1573-DV32P	83.62	14.64
		B1573-DV34P	79.2	10.22
	Prior art modification templates	B1573-DV30P	68.98	NA
		B1573-DV31P	70.20	NA
		B1573-DV21P	69.04	NA
		B1573-DV22P	70.80	NA

[0446] Table 21: HBeAg inhibition rates of unmodified sequence A1573 modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over prior art templates (%)
A1573	Modification templates of the present disclosure	A1573-DV29P	54.18	32.68
		B1573-DV26P	51.74	30.24
		B1573-DV27P	52.41	30.91
		B1573-DV28P	51.01	29.51
		B1573-DV32P	57.01	35.51
		B1573-DV34P	47.76	26.26
	Prior art modification templates	B1573-DV30P	21.50	NA
		B1573-DV31P	35.15	NA
		B1573-DV21P	33.55	NA
		B1573-DV22P	38.73	NA

[0447] When the unmodified sequence A1573 was modified using the templates DV26P-29P, DV32P, and DV34P of the present disclosure, the inhibition rates for both HBsAg and HBeAg were significantly improved compared to those modified with prior art templates. For

example, when modified with the template DV29P, the sequence A1573-DV29P showed increases in HBsAg and HBeAg inhibition rates by 16.08% and 32.68%, respectively. Detailed results are shown in Table 20 and Table 21.

[0448] (6) unmodified sequence B1575

[0449] Table 22: HBsAg inhibition rates of unmodified sequence B1575 modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B1575	Modification templates of the present disclosure	B1575-DV29P	91.78	31.19
		B1575-DV26P	91.68	31.09
		B1575-DV27P	90.82	30.23
		B1575-DV28P	72.07	11.48
		B1575-DV32P	90.1	29.51
		B1575-DV34P	84.92	24.33
	Prior art modification templates	B1575-DV30P	60.59	NA
		B1575-DV31P	70.37	NA
		B1575-DV21P	68.59	NA
		B1575-DV22P	66.48	NA

[0450] Table 23: HBeAg inhibition rates of unmodified sequence B1575 modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B1575	Modification templates of the present disclosure	B1575-DV29P	66.21	46.17
		B1575-DV26P	64.21	44.17
		B1575-DV27P	63.29	43.25
		B1575-DV28P	36.22	16.18
		B1575-DV32P	61.94	41.90
		B1575-DV34P	52.44	32.40
	Prior art modification templates	B1575-DV30P	20.04	NA
		B1575-DV31P	32.98	NA
		B1575-DV21P	31.35	NA
		B1575-DV22P	25.77	NA

[0451] When the unmodified sequence B1575 was modified using the templates DV26P-29P, DV32P, and DV34P of the present disclosure, the inhibition rates for both HBsAg and HBeAg were significantly increased compared to those modified with prior art templates. For example, when modified with the template DV29P, the sequence B1575-DV29P showed increases in HBsAg and HBeAg inhibition rates by 31.19% and 46.17%, respectively. Detailed results are shown in Table 22 and Table 23.

[0452] From this, the following conclusions can be drawn:

[0453] 1) The unmodified sequences B1572, A1573, and B1575, when modified with the templates DV26P-29P, DV32P and DV34P of the present disclosure, showed significantly superior inhibition of both HBsAg and HBeAg compared to those modified with prior art templates. For the unmodified sequence B1572, modification with the templates DV26P and DV27P of the present disclosure resulted in the highest inhibition rates for both HBsAg and HBeAg, significantly outperforming the prior art templates. For the unmodified sequences A1573 and B1575, modification with the template DV29P of the present disclosure resulted in the highest inhibition rates for both HBsAg and HBeAg, significantly outperforming the prior art templates.

[0454] 2) The activity of the same siRNA sequence can vary significantly when modified with different templates. For example, the unmodified sequence B1575 modified with the template DV29P of the present disclosure showed an increase in HBsAg inhibition rate by 29.99% compared to modification with the template DV28P of the present disclosure, indicating a significant improvement.

[0455] 3) In terms of siRNA with similar sequences, the sensitivity to different modification templates varies. For example, the unmodified sequence B1572 modified with the template DV27P of the present disclosure achieved the highest inhibition rates for both HBsAg and HBeAg, while the unmodified sequences A1573 and B1575 achieved the highest inhibition rates for HBsAg and HBeAg when modified with the template DV29P.

[0456] (7) unmodified sequence B205

[0457] Table 24: HBsAg inhibition rates of unmodified sequence B205 modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B205	Modification templates of the present disclosure	B205-DV29P	68.34	26.41
		B205-DV26P	75.67	33.74
		B205-DV27P	65.28	23.35
		B205-DV28P	78.48	36.55
		B205-DV32P	76.03	34.10
		B205-DV34P	76.35	34.42
	Prior art modification templates	B205-DV30P	48.57	NA
		B205-DV31P	59.55	NA
		B205-DV21P	44.86	NA
		B205-DV22P	41.93	NA

[0458] Table 25: HBeAg inhibition rates of unmodified sequence B205 modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B205	Modification templates of the present disclosure	B205-DV29P	35.59	25.00
		B205-DV26P	34.02	23.43
		B205-DV27P	30.57	19.98
		B205-DV28P	35.95	25.36
		B205-DV32P	34.96	24.37
		B205-DV34P	30.3	19.71
	Prior art modification templates	B205-DV30P	23.71	NA
		B205-DV31P	22.69	NA
		B205-DV21P	10.59	NA
		B205-DV22P	11.97	NA

[0459] When the unmodified sequence B205 was modified using the templates DV26P-29P, DV32P, and DV34P of the present disclosure, the inhibition rates for both HBsAg and HBeAg were significantly increased compared to those modified with prior art templates. For example, when modified with the template DV28P, the sequence B205-DV28P showed increases in HBsAg and HBeAg inhibition rates by 36.55% and 25.36%, respectively. Detailed results are shown in Table 24 and Table 25.

[0460] (8) unmodified sequence B207S

[0461] Table 26: HBsAg inhibition rates of unmodified sequence B207S modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B207S	Modification templates of the present disclosure	B207S-DV29P	92.11	35.37
		B207S-DV25P	87.34	30.60
		B207S-DV26P	81.17	24.43
		B207S-DV27P	87.52	30.78
		B207S-DV28P	82.1	25.36
		B207S-DV32P	91.72	34.98
		B207S-DV33P	86.65	29.91
		B207S-DV34P	81.63	24.89
	Prior art modification templates	B207S-DV30P	63.08	NA
		B207S-DV31P	70.31	NA
		B207S-DV21P	56.74	NA
		B207S-DV22P	57.75	NA

[0462] Table 27: HBeAg inhibition rates of unmodified sequence B207S modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B207S	Modification templates of the present disclosure	B207S-DV29P	55.18	26.71
		B207S-DV25P	43.85	15.38
		B207S-DV26P	45.81	17.34
		B207S-DV27P	45.77	17.30
		B207S-DV28P	45.55	17.08
		B207S-DV32P	47.42	18.95
		B207S-DV33P	46.85	18.38
		B207S-DV34P	40.38	11.91
	Prior art modification templates	B207S-DV30P	30.60	NA
		B207S-DV31P	33.35	NA
		B207S-DV21P	28.47	NA
		B207S-DV22P	30.55	NA

[0463] When the unmodified sequence B207S was modified using the templates DV25P-29P and DV32P-34P of the present disclosure, the inhibition rates for both HBsAg and HBeAg

were significantly improved compared to those modified with prior art templates. For example, when modified with the templates DV29P and DV32P, the sequence B207S-DV29P showed increases in HBsAg and HBeAg inhibition rates by 35.37% and 26.71%, respectively; the sequence B207S-DV32P showed increases in HBsAg and HBeAg inhibition rates by 34.98% and 18.95%. Detailed results are shown in Table 26 and Table 27.

[0464] (9) unmodified sequence B208

[0465] Table 28: HBsAg inhibition rates of unmodified sequence B208 modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B208	Modification templates of the present disclosure	B208-DV29P	46.27	15.67
		B208-DV26P	68.23	37.63
		B208-DV27P	54.29	23.69
		B208-DV28P	56.83	26.23
		B208-DV32P	42.13	11.53
		B208-DV34P	50.56	19.96
	Prior art modification templates	B208-DV30P	44.91	NA
		B208-DV31P	30.60	NA
		B208-DV21P	33.26	NA
		B208-DV22P	40.66	NA

[0466] Table 29: HBeAg inhibition rates of unmodified sequence B208 modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B208	Modification templates of the present disclosure	B208-DV29P	24.28	2.21
		B208-DV26P	41.45	19.38
		B208-DV27P	29.87	7.80
		B208-DV28P	25.44	3.37
		B208-DV32P	18.1	-3.97
		B208-DV34P	21.98	-0.09
	Prior art modification templates	B208-DV30P	31.71	NA
		B208-DV31P	23.35	NA
		B208-DV21P	22.07	NA

		B208-DV22P	29.90	NA
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[0467] The unmodified sequence B208 was modified using the templates DV26P-29P, DV32P, and DV34P of the present disclosure. Compared with those modified with prior art templates, the sequence B208-DV26P modified with the template DV26P showed the most significant improvement, with HBsAg and HBeAg inhibition rates increasing by 37.63% and 19.38%, respectively. Detailed results are shown in Table 28 and Table 29.

[0468] From this, the following conclusions can be drawn:

[0469] 1) For the unmodified sequences B205, B207S, and B208, modification with the templates DV25P-29P and DV32P-34P of the present disclosure resulted in significantly superior inhibition of both HBsAg and HBeAg compared to those modified with prior art templates. For the unmodified sequence B205, modification with the template DV28P of the present disclosure resulted in the highest inhibition rates for both HBsAg and HBeAg, significantly outperforming the prior art templates. For the unmodified sequence B207S, modification with the template DV29P of the present disclosure resulted in the highest inhibition rates for both HBsAg and HBeAg, significantly outperforming the prior art templates. For the unmodified sequence B208, modification with the template DV26P of the present disclosure resulted in the highest inhibition rates for both HBsAg and HBeAg, significantly outperforming the prior art templates.

[0470] 2) The activity of the same siRNA sequence can vary significantly when modified with different templates. For example, the unmodified sequence B208 modified with the template DV26P of the present disclosure showed an increase in HBsAg inhibition rate by 21.96% compared to modification with the template DV29P of the present disclosure, indicating a significant improvement.

[0471] (3) siRNA sequences with similar sequences can exhibit vastly different levels of activity. For example, the DV29P-modified sequence B207S-DV29P showed a 45.84% higher HBsAg inhibition rate compared to B208-DV29P, and a 23.77% higher inhibition rate compared to B205-DV29P.

[0472] 4) From this, it can be concluded that even for unmodified sequences with similar sequences, their sensitivity to different modification templates varies significantly. For example, the unmodified sequence B205 achieved the highest inhibition rates for HBsAg and

HBeAg (78.48% and 35.95%, respectively) when modified with the disclosed template DV28P; the unmodified sequence B207S achieved the highest inhibition rates for HBsAg and HBeAg (92.11% and 55.18%, respectively) when modified with the disclosed template DV29P; the unmodified sequence B208 achieved the highest inhibition rates for HBsAg and HBeAg (68.23% and 41.45%, respectively) when modified with the disclosed template DV26P.

[0473] (10) unmodified sequence A261

[0474] Table 30: HBsAg inhibition rates of unmodified sequence A261 modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
A261	Modification templates of the present disclosure	A261-DV29P	84.47	15.69
		B261-DV26P	85.6	16.82
		B261-DV27P	84.26	15.48
		B261-DV28P	81.34	12.56
		B261-DV32P	80.89	12.11
		B261-DV34P	82.06	13.28
	Prior art modification templates	B261-DV30P	68.78	NA
		B261-DV31P	72.86	NA
		B261-DV21P	76.75	NA
		B261-DV22P	69.62	NA

[0475] Table 31: HBeAg inhibition rates of unmodified sequence A261 modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over prior art templates (%)
A261	Modification templates of the present disclosure	A261-DV29P	23.83	13.40
		B261-DV26P	22.32	11.89
		B261-DV27P	26.27	15.84
		B261-DV28P	18.94	8.51
		B261-DV32P	22.46	12.03
		B261-DV34P	18.75	8.32
		B261-DV30P	10.43	NA
		B261-DV31P	19.60	NA

	Prior art modification templates	B261-DV21P	20.88	NA
		B261-DV22P	12.52	NA

[0476] When the unmodified sequence A261 was modified using the templates DV26P-29P, DV32P, and DV34P of the present disclosure, the inhibition rates for both HBsAg and HBeAg were significantly increased compared to those modified with prior art templates. For example, when modified with the template DV26P, the sequence B261-DV26P showed increases in HBsAg and HBeAg inhibition rates by 16.82% and 11.89%, respectively. Detailed results are shown in Table 30 and Table 31.

[0477] (11) unmodified sequence B262

[0478] Table 32: HBsAg inhibition rates of unmodified sequence B262 modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B262	Modification templates of the present disclosure	B262-DV29P	44.26	3.62
		B262-DV26P	68.98	28.34
		B262-DV27P	56.51	15.87
		B262-DV28P	69.63	28.99
		B262-DV32P	64.54	23.90
		B262-DV34P	64.63	23.99
	Prior art modification templates	B262-DV30P	40.64	NA
		B262-DV31P	42.47	NA
		B262-DV21P	41.43	NA
		B262-DV22P	43.94	NA

[0479] Table 33: HBeAg inhibition rates of unmodified sequence B262 modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B262	Modification templates of the	B262-DV29P	15.39	7.14
		B262-DV26P	26.01	17.76
		B262-DV27P	22.86	14.61

	present disclosure	B262-DV28P	26.18	17.93
		B262-DV32P	24.24	15.99
		B262-DV34P	19.67	11.42
	Prior art modification templates	B262-DV30P	9.48	NA
		B262-DV31P	10.25	NA
		B262-DV21P	9.63	NA
		B262-DV22P	11.13	NA

[0480] When the unmodified sequence B262 was modified using the templates DV26P-29P, DV32P, and DV34P of the present disclosure, the inhibition rates for both HBsAg and HBeAg were significantly increased compared to those modified with prior art templates. For example, when modified with the template DV26P, the sequence B261-DV26P showed increases in HBsAg and HBeAg inhibition rates by 28.99% and 17.93%, at a maximum. Detailed results are shown in Table 32 and Table 33.

[0481] (12) unmodified sequence B264

[0482] Table 34: HBsAg inhibition rates of unmodified sequence B264 modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B264	Modification templates of the present disclosure	B264-DV29P	54.01	35.22
		B264-DV26P	43.99	25.20
		B264-DV27P	33.85	15.06
		B264-DV28P	34.32	15.53
		B264-DV32P	40.74	21.95
		B264-DV34P	49.25	30.46
	Prior art modification templates	B264-DV30P	27.46	NA
		B264-DV31P	22.99	NA
		B264-DV21P	21.70	NA
		B264-DV22P	18.79	NA

Table 35: HBeAg inhibition rates of unmodified sequence B264 modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over
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				prior art templates (%)
B264	Modification templates of the present disclosure	B264-DV29P	14.65	13.12
		B264-DV26P	13.12	11.59
		B264-DV27P	9.23	7.70
		B264-DV28P	9.96	8.43
		B264-DV32P	11.31	9.78
		B264-DV34P	14.16	12.63
	Prior art modification templates	B264-DV30P	6.40	NA
		B264-DV31P	5.44	NA
		B264-DV21P	3.19	NA
		B264-DV22P	1.53	NA

[0483] When the unmodified sequence B264 was modified using the templates DV26P-29P, DV32P, and DV34P of the present disclosure, the inhibition rates for both HBsAg and HBeAg were significantly increased compared to those modified with prior art templates. For example, when modified with the template DV29P, the sequence B264-DV29P showed increases in HBsAg and HBeAg inhibition rates by 35.22% and 13.12%, respectively. Detailed results are shown in Table 34 and Table 35.

[0484] From this, the following conclusions can be drawn:

[0485] 1) For the unmodified sequences A261, B262, and B264, modification with the templates DV26P-29P, DV32P and DV34P of the present disclosure resulted in significantly superior inhibition of both HBsAg and HBeAg compared to those modified with prior art templates. For the unmodified sequence A261, modification with the template DV26P of the present disclosure resulted in the highest inhibition rates for HBsAg and modification with the template DV27P resulted in the highest inhibition rates for HBeAg, significantly outperforming the prior art templates. For the unmodified sequence B262, modification with the template DV28P of the present disclosure resulted in the highest inhibition rates for both HBsAg and HBeAg, significantly outperforming the prior art templates. For the unmodified sequence B264, modification with the template DV29P of the present disclosure resulted in the highest inhibition rates for both HBsAg and HBeAg, significantly outperforming the prior art templates.

[0486] 2) The activity of the same siRNA sequence can vary significantly when modified with different templates. For example, the unmodified sequence B264 modified with the template DV27P of the present disclosure showed an decrease in HBsAg inhibition rate by

20.16% compared to modification with the template DV29P, indicating a significant drop.

[0487] (3) siRNA sequences with similar sequences can exhibit vastly different levels of activity. For example, the DV29P-modified sequence A261-DV29P showed a 40.21% higher HBsAg inhibition rate compared to B262-DV29P, and a 30.46% higher inhibition rate compared to B264-DV29P.

[0488] 4) From this, it can be concluded that despite having similar unmodified sequences, the sensitivity to different modification templates varies. For example, the unmodified sequence B262 modified with the template DV28P of the present disclosure achieved the highest inhibition rates for both HBsAg and HBeAg, while the unmodified sequence B264 achieved the highest inhibition rates for HBsAg and HBeAg when modified with the template DV29P.

[0489] (13) unmodified sequence A416

[0490] Table 36: HBsAg inhibition rates of unmodified sequence A416 modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
A416	Modification templates of the present disclosure	A416-DV29P	79.71	22.02
		B416-DV26P	75.18	17.49
		B416-DV27P	86.88	29.19
		B416-DV28P	74.34	16.65
		B416-DV32P	70.09	12.40
		B416-DV34P	79.83	22.14
	Prior art modification templates	B416-DV30P	58.66	NA
		B416-DV31P	62.28	NA
		B416-DV21P	57.69	NA
		B416-DV22P	59.74	NA

[0491] Table 37: HBeAg inhibition rates of unmodified sequence A416 modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over
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				prior art templates (%)
A416	Modification templates of the present disclosure	A416-DV29P	43.72	25.31
		B416-DV26P	40.96	22.55
		B416-DV27P	52.24	33.83
		B416-DV28P	40.23	21.82
		B416-DV32P	34.14	15.73
		B416-DV34P	40.65	22.24
	Prior art modification templates	B416-DV30P	18.80	NA
		B416-DV31P	23.19	NA
		B416-DV21P	18.41	NA
		B416-DV22P	21.15	NA

[0492] When the unmodified sequence A416 was modified using the templates DV26P-29P, DV32P, and DV34P of the present disclosure, the inhibition rates for both HBsAg and HBeAg were significantly increased compared to those modified with prior art templates. For example, when modified with DV27P, the sequence showed increases in HBsAg and HBeAg inhibition rates by 29.19% and 33.83%, respectively. Detailed results are shown in Table 36 and Table 37.

[0493] (14) unmodified sequence B416S

[0494] Table 38: HBsAg inhibition rates of unmodified sequence B416S modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B416S	Modification templates of the present disclosure	B416S-DV29P	7.30	0.92
		B416S-DV26P	12.43	6.05
		B416S-DV27P	13.62	7.24
		B416S-DV28P	10.78	4.40
		B416S-DV32P	13.64	7.26
		B416S-DV34P	8.56	2.18
	Prior art modification templates	B416S-DV30P	6.38	NA
		B416S-DV31P	6.98	NA
		B416S-DV21P	8.85	NA
		B416S-DV22P	10.44	NA

[0495] Table 39: HBeAg inhibition rates of unmodified sequence B416S modified with templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B416S	Modification templates of the present disclosure	B416S-DV29P	10.64	13.39
		B416S-DV26P	16.35	19.10
		B416S-DV27P	22.62	25.37
		B416S-DV28P	7.76	10.51
		B416S-DV32P	4.86	7.61
		B416S-DV34P	1.11	3.86
	Prior art modification templates	B416S-DV30P	-2.75	NA
		B416S-DV31P	6.43	NA
		B416S-DV21P	10.16	NA
		B416S-DV22P	10.67	NA

[0496] The unmodified sequence B416S was modified using the templates DV26P-29P, DV32P, and DV34P of the present disclosure. Compared with those modified with prior art templates, the above sequences showed relatively low inhibition rates for both HBsAg and HBeAg, which were less than 25%. Detailed results are shown in Table 38 and Table 39.

[0497] (15) unmodified sequence B418S

[0498] Table 40: HBsAg inhibition rates of unmodified sequence B418S modified with templates

unmodified sequence code	Modification template	Sequence code	HBsAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B418S	Modification templates of the present disclosure	B418S-DV29P	82.05	20.31
		B418S-DV26P	87.20	25.46
		B418S-DV27P	78.23	16.49
		B418S-DV28P	84.79	23.05
		B418S-DV32P	84.77	23.03
		B418S-DV34P	87.36	25.62
	Prior art modification templates	B418S-DV30P	61.74	NA
		B418S-DV31P	74.66	NA
		B418S-DV21P	71.43	NA
		B418S-DV22P	73.83	NA

[0499] Table 41: HBeAg inhibition rates of unmodified sequence B418S modified with

templates

unmodified sequence code	Modification template	Sequence code	HBeAg inhibition rate (%)	Maximum improvement over prior art templates (%)
B418S	Modification templates of the present disclosure	B418S-DV29P	45.48	17.47
		B418S-DV26P	53.70	25.69
		B418S-DV27P	40.78	12.77
		B418S-DV28P	51.16	23.15
		B418S-DV32P	49.10	21.09
		B418S-DV34P	54.92	26.91
	Prior art modification templates	B418S-DV30P	28.01	NA
		B418S-DV31P	35.26	NA
		B418S-DV21P	32.32	NA
		B418S-DV22P	33.63	NA

[0500] When the unmodified sequence B418S was modified using the templates DV26P-29P, DV32P, and DV34P of the present disclosure, the inhibition rates for both HBsAg and HBeAg were significantly increased compared to those modified with prior art templates. For example, when modified with the template DV34P, the sequence showed increases in HBsAg and HBeAg inhibition rates by 25.62% and 26.91%, respectively. Detailed results are shown in Table 40 and Table 41.

[0501] From this, the following conclusions can be drawn:

[0502] 1) The unmodified sequences A416 and B418S, when modified with the templates DV26P-29P, DV32P and DV34P of the present disclosure, showed significantly superior inhibition of both HBsAg and HBeAg compared to those modified with prior art templates. For the unmodified sequence A416, modification with the template DV27P of the present disclosure resulted in the highest inhibition rates for both HBsAg and HBeAg. For the unmodified sequence B418S, modification with DV34P resulted in the highest inhibition rates for both HBsAg and HBeAg. For the unmodified sequence B416S, modification with DV26P-28P, DV32P, DV34P, and the sequence modified by modified templates of prior art resulted in relatively low inhibition rates for both HBsAg and HBeAg, which were less than 25%.

[0503] 2) The activity of the same siRNA sequence can vary significantly when modified with different templates. For example, the unmodified sequence A416 modified with the

template DV27P of the present disclosure showed an decrease in HBsAg inhibition rate by 16.79% compared to the template DV32P.

[0504] (3) siRNA sequences with similar sequences can exhibit vastly different levels of activity. For example, the DV29P-modified sequence A416-DV29P showed a 72.41% higher HBsAg inhibition rate compared to B416S-DV29P.

[0505] 4) From this, it can be concluded that despite having similar unmodified sequences, the sensitivity to different modification templates varies. For example, the unmodified sequence A416 modified with the template DV27P of the present disclosure achieved the highest inhibition rates for both HBsAg and HBeAg, while the unmodified sequence B418S achieved the highest inhibition rates for HBsAg and HBeAg when modified with the template DV34P.

[0506] (ii) IC₅₀ experiment and cytotoxicity results

[0507] The top-performing sequences from the single-concentration screening experiment were selected for IC₅₀ experiments, including B207S-DV29P, B207S-DV32P, B1575-DV29P, B1575-DV26P, B1575-DV27P, B1572-DV27P, B1573-DV29P, B1550-DV27P, B261-DV26P, B416-DV27P, B418S-DV34P, and the positive control APC-VIR, totaling 12 sequences. The IC₅₀ concentrations of each sequence were determined.

[0508] Table 42: IC₅₀ experimental results for modified sequences

Sequence code	IC ₅₀ (nM)	IC ₅₀ (nM)	CC ₅₀ (nM)	Cell viability at 10 nM (%)
	HBsAg	HBeAg		
B1572-DV27P	0.0538	0.2073	>10	111.87
B1550-DV27P	0.0570	0.2143	>10	109.67
B207S-DV32P	0.0572	0.7848	>10	111.97
B1575-DV26P	0.0629	0.2923	>10	95.97
B1575-DV27P	0.0664	0.3226	>10	91.47
B261-DV26P	0.0859	3.5030	>10	98.89
B1575-DV29P	0.1134	0.4648	>10	94.89
B1573-DV29P	0.1191	0.5772	>10	92.92
B418S-DV34P	0.1198	0.4498	>10	100.53
B207S-DV29P	0.1292	0.8175	>10	106.15
B416-DV27P	0.1321	0.4353	>10	99.97
APC-VIR	0.3362	1.8900	>10	106.05

[0509] The IC₅₀ experimental results showed that the IC₅₀ values of these 11 sequences for

HBsAg ranged from 0.05 nM to 0.15 nM, all of which were lower than the IC₅₀ value of the positive control sequence (0.3362 nM), demonstrating superior inhibitory effects compared to the positive control. For example, the IC₅₀ values of sequences B1572-DV27P, B1550-DV27P, and B207S-DV32P for HBsAg were 0.0538 nM, 0.0570 nM, and 0.0572 nM, respectively, indicating that these sequences effectively inhibit HBsAg expression at low concentrations. Except for the sequence B261-DV26P, the IC₅₀ values of the remaining 10 sequences for HBeAg ranged from 0.2 nM to 0.85 nM, all of which were lower than the IC₅₀ value of the positive control sequence (1.89 nM), demonstrating superior inhibitory effects compared to the positive control. For example, the IC₅₀ values of sequences B1550-DV27P, B1572-DV27P, and B1575-DV26P for HBeAg were 0.2073 nM, 0.2143 nM, and 0.2923 nM, respectively, indicating that these sequences effectively inhibit HBeAg expression at low concentrations. Experimental results are shown in Table 42.

[0510] The cytotoxicity results showed that all 11 sequences exhibited cell viability greater than 90% at the highest tested concentration of 10 nM, indicating no significant cytotoxicity and suggesting a wide concentration range for selection. Detailed results are shown in Table 42.

[0511] Example 5: Comparison of HBV gene inhibition with prior art sequences

[0512] This example compares the inhibition efficiency of HBV genes between: unmodified sequences disclosed in prior art that are identical or similar to the disclosed unmodified sequences B207S, B1575, B1550, B1572, A416, B1573, and B418S; and sequences modified with prior art disclosed templates (DV30P, DV31P, DV21P, DV22P) and modified with the templates of the present disclosure (DV25P, DV26P, DV27P, DV28P, DV29P, DV32P, DV33P, and DV34P).

[0513] 1. Experimental materials

[0514] Test samples:

[0515] (1) Sequences disclosed in prior art, as listed in Table 43.

[0516] Table 43: Sequences disclosed in prior art

Sequence code	Position	Sense strand	Sequence ID No	Antisense strand	Sequence ID No	Source
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P206	206-226	CGGGGUUUUUC UUGUUGACAA	19	UUGUCAACAAGA AAAACCCCG	76	WO2023093896A1
B207S	207-226	GGGGUUUUUCU UGUUGACAA	1	UUGUCAACAAGA AAAACCCCGC	8	Sequences designed in the present disclosure
P413	413-433	UCCUGCUGCUA UGCCUCAUCU	27	AUGAGGCAUAGC AGCAGGATT	77	US20060287266A1
A416	416-436	UGCUGCUAUGC CUCAUCUUCU	7	AGAAGAUGAGGC AUAGCAGCAGG	14	Sequences designed in the present disclosure
P1551	1551-1571	CCGUCUGUGCC UUCUCAUCUA	44	UAGAUGAGAAGG CACAGACGGGG	78	CN108064294B
A1550	1550-1570	CCCGUCUGUGC CUUCUCAUCU	2	AGAUGAGAAGGC ACAGACGGGGA	9	Sequences designed in the present disclosure
APC	1579-1597	GUGUGCACUUC GCUUCACA	45	UGUGAAGCGAAG UGCACACUU	79	CN114828859A
B1575	1575-1595	GACCGUGUGCA CUUCGCUUCA	4	UGAAGCGAAGUG CACACGGUCCG	11	Sequences designed in the present disclosure
A1573	1573-1593	CGGACCGUGUG CACUUCGCUU	5	AAGCGAAGUGCA CACGGUCCGGC	12	Sequences designed in the present disclosure
B1572	1572-1592	CCGGACCGUGU GCACUUCGCU	3	AGCGAAGUGCAC ACGGUCCGGCA	10	Sequences designed in the present disclosure

[0517] Note: The sequences disclosed in the above patent applications are all unmodified RNA sequences.

[0518] (2) The siRNA sequences modified with the templates DV25P, DV26P, DV27P, DV28P, DV29P, DV32P, DV33P, DV34P, DV30P, DV31P, DV21P, and DV22P, as described in Example 4, along with their corresponding unmodified sequences.

[0519] Cell line: HepG2.2.15 cells.

[0520] Vehicle reagents: Sterile nuclease-free water and Gibco™ Opti-MEM.

[0521] 2. Experimental methods

[0522] Referring to Example 2, the concentration for the single-concentration screening experiment was set at 0.3 nM.

[0523] 3. Experimental results

[0524] The experimental results showed that the unmodified sequences disclosed in this

study—B207S, A1550, B1572, B1575, A1573, B418S, and A416—exhibited significantly higher inhibition rates for both HBsAg and HBeAg compared to the similar sequences P206, P413, P1551, and APC from prior art. After template modification, the inhibition rates were further significantly improved.

[0525] The comparison of inhibition rates for HBsAg and HBeAg between sequences which have similar sequences to the present disclosure disclosed in prior art and those disclosed in the present disclosure are shown in Table 44 and Table 45.

[0526] From Table 43, it can be observed that, compared to the prior art sequence P206, the disclosed sequence B207S is shifted backward by 1 base and shortened by 1 base. Compared with P206, B207S showed an increase in inhibition rates for HBsAg and HBeAg by 15.57% and 11.69%, respectively. When B207S was further modified with the disclosed templates, the inhibition efficiency was further enhanced. For example, when modified with DV32P, the sequence B207S-DV32P showed an increase in inhibition rates for HBsAg and HBeAg by 28.92% and 20.55%, respectively, significantly outperforming the prior art modification templates (DV30P, DV31P, DV21P, and DV22P).

[0527] Compared to the prior art sequence P413, the disclosed sequence A416 is shifted backward by 3 bases. Compared with P413, A416 showed an increase in inhibition rates for HBsAg and HBeAg by 5.95% and 7.42%, respectively. When A416 was further modified with the disclosed templates, the inhibition efficiency was further enhanced. For example, when modified with DV27P, the sequence B416-DV27P showed an increase in inhibition rates for HBsAg and HBeAg by 24.16% and 21.26%, respectively, significantly outperforming the prior art modification templates (DV30P, DV31P, DV21P, and DV22P).

[0528] Compared to the prior art sequence P1551, the disclosed sequence A1550 is shifted forward by 1 base. Compared to P1551, A1550 showed an increase in inhibition rates for HBsAg and HBeAg by 7.98% and 9.03%, respectively. When A1550 was further modified with the disclosed templates, the inhibition efficiency was further enhanced. For example, when modified with DV27P, the sequence B1550-DV27P showed an increase in inhibition rates for HBsAg and HBeAg by 23.96% and 26.06%, respectively, significantly outperforming the prior art modification templates (DV30P, DV31P, DV21P, and DV22P).

[0529] Compared to the prior art sequence APC, the disclosed sequences B1575, B1573, and

B1572 are shifted forward by 4, 6, and 7 bases, respectively, and all are extended by 2 bases in length. Compared to APC, B1575, B1573, and B1572 showed an increase in inhibition rates for HBsAg by 13.46%, 10.80%, and 9.94%, respectively. When modified with the disclosed templates, the inhibition efficiency was further improved. For example, the sequence B1575-DV29P showed the most significant improvement, with an increase in HBsAg inhibition rate by 29.28% and an increase in HBeAg inhibition rate by 24.25%. The sequence B1575-DV26P showed an increase in HBsAg inhibition rate by 27.18% and an increase in HBeAg inhibition rate by 16.55%, significantly outperforming the prior art modification templates (DV30P, DV31P, DV21P, and DV22P).

[0530] Table 44: Comparison of HBsAg inhibition rates between prior art disclosed sequences and sequences of the present disclosure

Prior art disclosed sequences		Sequences (unmodified) of the present disclosure			Sequences modified with templates of the present disclosure		
Sequence code	HBsAg inhibition rate (%)	Sequence code	HBsAg inhibition rate (%)	Improvement over prior art (%)	Sequence code	HBsAg inhibition rate (%)	Improvement over unmodified sequences of the present disclosure (%)
P206	53.77	B207S	69.34	15.57	B207S-DV25P	94.78	25.44
					B207S-DV26P	86.62	17.28
					B207S-DV27P	95.06	25.72
					B207S-DV28P	86.52	17.18
					B207S-DV29P	98.65	29.31
					B207S-DV32P	98.26	28.92
					B207S-DV33P	88.32	18.98
					B207S-DV34P	86.63	17.29
					B207S-DV30P	74.85	5.51
					B207S-DV31P	81.37	12.03
					B207S-DV21P	69.82	0.48
					B207S-DV22P	71.01	1.67
P413	52.04	A416	57.99	5.95	B416-DV26P	75.33	17.34
					B416-DV27P	82.15	24.16
					B416-DV28P	75.07	17.08
					A416-DV29P	75.70	17.71

					B416-DV32P	70.66	12.67
					B416-DV34P	81.69	23.70
					B416-DV30P	56.21	-1.78
					B416-DV31P	69.48	11.49
					B416-DV21P	58.99	1.00
					B416-DV22P	60.95	2.96
P1551	57.31	A1550	65.29	7.98	B1550-DV26P	88.52	23.23
					B1550-DV27P	89.25	23.96
					B1550-DV28P	77.80	12.51
					A1550-DV29P	86.74	21.45
					B1550-DV32P	77.73	12.44
					B1550-DV33P	79.47	14.18
					B1550-DV34P	85.48	20.19
					B1550-DV25P	85.81	20.52
					B1550-DV30P	68.12	2.83
					B1550-DV31P	63.80	-1.49
					B1550-DV21P	64.15	-1.14
					B1550-DV22P	68.90	3.61
APC	50.43	B1575	63.89	13.46	B1575-DV26P	91.07	27.18
					B1575-DV27P	88.25	24.36
					B1575-DV28P	72.83	8.94
					B1575-DV29P	93.17	29.28
					B1575-DV32P	87.32	23.43
					B1575-DV34P	82.93	19.04
					B1575-DV30P	59.71	-4.18
					B1575-DV31P	72.68	8.79
					B1575-DV21P	65.74	1.85
					B1575-DV22P	64.72	0.83
		A1573	61.23	10.80	B1573-DV26P	81.64	20.41
					B1573-DV27P	84.86	23.63
					B1573-DV28P	83.80	22.57
					A1573-DV29P	85.81	24.58
					B1573-DV32P	84.80	23.57
					B1573-DV34P	80.26	19.03
					B1573-DV30P	63.12	1.89
					B1573-DV31P	63.19	1.96
					B1573-DV21P	65.81	4.58
					B1573-DV22P	66.99	5.76
		B1572	60.37	9.94	B1572-DV26P	86.77	26.40
					B1572-DV27P	89.82	29.45
					B1572-DV28P	83.89	23.52
					B1572-DV29P	84.17	23.80
					B1572-DV32P	81.39	21.02

					B1572-DV34P	83.96	23.59
					B1572-DV30P	66.54	6.17
					B1572-DV31P	65.84	5.47
					B1572-DV21P	69.99	9.62
					B1572-DV22P	71.53	11.16

[0531] Table 45: Comparison of HBeAg inhibition rates between prior art disclosed sequences and sequences of the present disclosure

Prior art disclosed sequences		Sequences (unmodified) of the present disclosure			Sequences modified with templates of the present disclosure		
Sequence code	HBeAg inhibition rate (%)	Sequence code	HBeAg inhibition rate (%)	Improvement over prior art (%)	Sequence code	HBeAg inhibition rate (%)	Improvement over sequences of the present disclosure (%)
P206	27.19	B207S	38.88	11.69	B207S-DV25P	41.66	2.78
					B207S-DV26P	47.89	9.01
					B207S-DV27P	46.54	7.66
					B207S-DV28P	47.63	8.75
					B207S-DV29P	58.42	19.54
					B207S-DV32P	59.43	20.55
					B207S-DV33P	47.63	8.75
					B207S-DV34P	38.69	-0.19
					B207S-DV30P	34.25	-4.63
					B207S-DV31P	40.94	2.06
					B207S-DV21P	30.74	-8.14
					B207S-DV22P	31.84	-7.04
P413	16.93	A416	24.35	7.42	B416-DV26P	41.99	17.64
					B416-DV27P	45.61	21.26
					B416-DV28P	42.71	18.36
					A416-DV29P	43.65	19.30
					B416-DV32P	37.06	12.71
					B416-DV34P	39.47	15.12
					B416-DV30P	20.97	-3.38
					B416-DV31P	25.68	1.33
					B416-DV21P	20.85	-3.50
					B416-DV22P	23.80	-0.55
P1551	18.05	A1550	27.08	9.03	B1550-DV26P	48.71	21.63
					B1550-DV27P	53.14	26.06
					B1550-DV28P	38.04	10.96
					A1550-DV29P	45.43	18.35
					B1550-DV32P	41.80	14.72

					B1550-DV33P	45.16	18.08
					B1550-DV34P	46.20	19.12
					B1550-DV25P	46.53	19.45
					B1550-DV30P	31.30	4.22
					B1550-DV31P	30.55	3.47
					B1550-DV21P	30.09	3.01
					B1550-DV22P	33.10	6.02
APC	29.92	B1575	44.16	14.24	B1575-DV26P	60.71	16.55
					B1575-DV27P	55.11	10.95
					B1575-DV28P	39.88	-4.28
					B1575-DV29P	68.41	24.25
					B1575-DV32P	53.41	9.25
					B1575-DV34P	52.81	8.65
					B1575-DV30P	41.39	-2.77
					B1575-DV31P	49.74	5.58
					B1575-DV21P	43.74	-0.42
					B1575-DV22P	42.29	-1.87
		A1573	29.76	-0.16	B1573-DV26P	51.92	22.16
					B1573-DV27P	52.11	22.35
					B1573-DV28P	48.66	18.90
					A1573-DV29P	53.09	23.33
					B1573-DV32P	58.01	28.25
					B1573-DV34P	41.95	12.19
					B1573-DV30P	22.65	-7.11
					B1573-DV31P	32.32	2.56
					B1573-DV21P	30.80	1.04
					B1573-DV22P	39.54	9.78
		B1572	35.63	5.71	B1572-DV26P	60.82	25.19
					B1572-DV27P	61.66	26.03
					B1572-DV28P	53.00	17.37
					B1572-DV29P	55.21	19.58
					B1572-DV32P	39.45	3.82
					B1572-DV34P	48.64	13.01
					B1572-DV30P	35.69	0.06
					B1572-DV31P	29.89	-5.74
					B1572-DV21P	35.69	0.06
					B1572-DV22P	37.37	1.74

[0532] Example 6: Inhibition of HBV and off-target genes by sequences with specific anti-off-target design

[0533] In practical applications of siRNA, there are numerous instances where non-target

mRNAs with only partial complementarity to the guide strand (antisense strand) are also suppressed. Studies by Alnylam Pharmaceuticals have shown that the hepatotoxicity of GalNAc-conjugated siRNA is primarily attributed to off-target effects caused by the suppression of incorrect targets through a miRNA-like recognition mechanism.

[0534] To address this issue, thermolabile nucleotide modifications, such as glycol nucleic acid (GNA), unlocked nucleic acid (UNA), or DNA, can be introduced at positions 6 and 7 of the seed region of the antisense strand. These modifications disrupt the seed region of the antisense strand, thereby reducing the binding of siRNA to non-target mRNAs through seed region recognition and significantly mitigating off-target effects and hepatotoxicity. In its latest fifth-generation template design, Alnylam Pharmaceuticals has adopted the approach of placing a GNA modification at position 7 of the siRNA antisense strand to reduce off-target effects.

[0535] In this example, the unmodified sequences B207S, B1575, A1550, B1572, A1573, A261, A416, and B418S were used to study off-target and anti-off-target effects. To evaluate the off-target effects of each sequence, qPCR was used to compare the inhibition efficiency of target genes and off-target genes in cellular experiments.

[0536] To reduce off-target effects, sequences with potential off-target activity, such as B418S-DV34P and B416-DV27P, were modified with DNA or GNA at positions 6 and 7 of the antisense strand. These anti-off-target modifications were compared with unmodified sequences in terms of activity and off-target effects.

[0537] 1. Experimental materials

[0538] Test samples: sequences modified with templates listed in Table 46 and corresponding sequences with anti-off-target designs (synthesis methods refer to Example 1). In the sequence codes, d67B indicates that the sequence is modified with the d67B anti-off-target design, d7B indicates modification with the d7B anti-off-target design, and + indicates modification with GNA for anti-off-target purposes.

[0539] Cell line: HepG2.2.15 cells.

[0540] Vehicle reagents: Sterile nuclease-free water and Gibco™ Opti-MEM™.

[0541] Table 46: Template-modified and anti-off-target designed sequences

Unmodified sequence code	Sequence code	Sense strand sequence 5'-3'	Sequence ID No	Unmodified sequence ID No	Antisense strand sequence 5'-3'	Sequence ID No	Unmodified sequence ID No
B418S	B418S-DV3 4P	Cms-Ums-Gm-Cm-Um-Af-Um-Gf-Cm-Cf-Um-Cm-Am-Um-Cm-Uf-Um-Cm-Um-Um	106	6	AmsEVP-Afs-Gf-Am-Am-Gf-Am-Um-Gm-Am-Gm-Gm-Cm-Af-Um-Af-Gm-Cm-Am-Gms-Cms-Am	216	13
B418S	C418S-DV3 4Pd6 7B	Cms-Ums-Gm-Cm-Um-Af-Um-Gf-Cm-Cf-Um-Cm-Am-dT-dC-Uf-Um-Cm-Um-Um	305	6	AmsEVP-Afs-Gf-Am-Am-dG-dA-Um-Gm-Am-Gm-Gm-Cm-Af-Um-Af-Gm-Cm-Am-Gms-Cms-Am	320	13
B418S	C418S-DV3 4Pd7 B	Cms-Ums-Gm-Cm-Um-Af-Um-Gf-Cm-Cf-Um-Cm-Am-dT-Cm-Uf-Um-Cm-Um-Um	306	6	AmsEVP-Afs-Gf-Am-Am-Gf-dA-Um-Gm-Am-Gm-Gm-Cm-Af-Um-Af-Gm-Cm-Am-Gms-Cms-Am	321	13
B418S	C418S-DV3 4P+	Cms-Ums-Gm-Cm-Um-Af-Um-Gf-Cm-Cf-Um-Cm-Am-Um-Cm-Uf-Um-Cm-Um-Um	307	6	AmsEVP-Afs-Gf-Am-Am-G(gna)-Am-Um-Gm-Am-Gm-Gm-Cm-Af-Um-Af-Gm-Cm-Am-Gms-Cms-Am	322	13
A416	B416-DV2 7P	Ums-Gms-Cm-Um-Gm-Cm-Uf-Am-Uf-Gf-Cf-Cm-Um-Cm-Am-Um-Cf-Um-Um-Cm-Um	112	7	AmsEVP-Gfs-Am-Af-Gf-Af-Um-Gm-Am-Gm-Gm-Cm-Am-Uf-Am-Gf-Cm-Am-Gm-Cm-Ams-Gms-Gm	219	14
A416	C416-DV2 7Pd6 7B	Ums-Gms-Cm-Um-Gm-Cm-Uf-Am-Uf-Gf-Cf-Cm-Um-Cm-dA-dT-Cf-Um-Um-Cm-Um	302	7	AmsEVP-Gfs-Am-Af-Gf-dA-dT-Gm-Am-Gm-Gm-Cm-Am-Uf-Am-Gf-Cm-Am-Gm-Cm-Ams-Gms-Gm	317	14
A416	C416-DV2 7Pd7 B	Ums-Gms-Cm-Um-Gm-Cm-Uf-Am-Uf-Gf-Cf-Cm-Um-Cm-dA-Um-Cf-Um-Um-Cm-Um	303	7	AmsEVP-Gfs-Am-Af-Gf-Af-dT-Gm-Am-Gm-Gm-Cm-Am-Uf-Am-Gf-Cm-Am-Gm-Cm-Ams-Gms-Gm	318	14
A416	C416-DV2 7P+	Ums-Gms-Cm-Um-Gm-Cm-Uf-Am-Uf-Gf-Cf-Cm-Um-Cm-Am-Um-Cf-Um-Um-Cm-Um	304	7	AmsEVP-Gfs-Am-Af-Gf-A(gna)-Um-Gm-Am-Gm-Gm-Cm-Am-Uf-Am-Gf-Cm-Am-Gm-Cm-Ams-Gms-Gm	319	14

[0542] 2. Experimental methods

[0543] The inhibition of HBV and off-target genes in HepG2.2.15 cells by the test samples

was detected using qRT-PCR.

[0544] 2.1 Cell culture

[0545] Subculture: HepG2.2.15 cells were subcultured in DMEM/F12 medium containing 10% fetal bovine serum, 370 µg/mL Geneticin, 1% L-glutamine, 1% non-essential amino acids, and 1% penicillin-streptomycin. The cells were maintained in a cell incubator at 37°C with 5% CO₂ and subcultured every three days. For subculturing, the cells were detached using 0.25% trypsin, centrifuged at 800 rpm for 3 minutes, and the supernatant was discarded. Fresh medium was then added for further subculture.

[0546] Plating: HepG2.2.15 cells were plated in DMEM/F12 medium containing 10% fetal bovine serum (FBS), 1% L-glutamine, 1% non-essential amino acids, and 1% penicillin-streptomycin.

[0547] 2.2 Cell transfection

[0548] Preparation of transfection reagent: Lipofectamine RNAiMAX and Opti-MEM were mixed in a 2:98 ratio and vortexed thoroughly.

[0549] Preparation of transfection complex: 30 µL of siRNA solution diluted in Opti-MEM was combined with 30 µL of the transfection mixture at a 1:1 (v/v) ratio, vortexed, and incubated at room temperature for 15 minutes to form the transfection complex.

[0550] Preparation of control transfection reagent: 15 µL of the prepared transfection mixture was added to 15 µL of Opti-MEM. The mixture was vortexed and then incubated at room temperature for 15 minutes.

[0551] The prepared transfection complexes were added to a 96-well cell culture plate (15 µL per well, with three replicates per sequence), resulting in final siRNA concentrations of 0.065 nM, 0.14 nM, and 0.28 nM per well. For each siRNA, three concentrations were tested, with three replicates per concentration. 135 µL of cell suspension (containing 2.25×10^4 cells) was added to each well. After mixing using the cross-mixing method, the plate was placed in a 37°C, 5% CO₂ cell incubator for incubation.

[0552] 2.3 RNA extraction and reverse transcription

[0553] After 48 hours of transfection, the medium was removed, and the cells were collected for RNA extraction. Total RNA was extracted using the RNeasy® 96 Kit (QIAGEN-74182) according to the manufacturer's instructions. Subsequently, RNA was reverse-transcribed

into cDNA using the HiScript III RT SuperMix for qPCR (Vazyme) following the manufacturer's protocol.

[0554] 2.4 RT-qPCR

[0555] The cDNA of HBV and off-target genes was detected by qPCR, with GAPDH cDNA used as an internal control for parallel detection. A total of 8 µL of prepared qPCR reaction mix and 2 µL of sample cDNA were added to each well of a 384-well plate. The SYBR qPCR reaction program was as follows: incubation at 50°C for 2 min, heating at 95°C for 2 min, followed by cycling at 95°C for 5 s and 60°C for 30 s for a total of 40 cycles. Finally, the melting curve was analyzed by heating at 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s.

[0556] 2.5 Data analysis

[0557] The RNA expression levels of the target genes in the samples were calculated based on the CT values using the $\Delta\Delta CT$ relative quantification method. The relative expression of the target genes was expressed as $2^{-\Delta\Delta CT}$.

[0558] The calculation formulas are as follows:

[0559] $\Delta CT = \text{Average CT value of the target gene} - \text{Average CT value of GAPDH};$

[0560] $\Delta\Delta CT = \Delta CT (\text{experimental group}) - \Delta CT (\text{transfection control group});$

[0561] Relative mRNA expression of the target gene = $2^{-\Delta\Delta CT};$

[0562] Gene expression inhibition rate (%) = $(1 - \text{mRNA expression level of the sample} / \text{mRNA expression level of the transfection control group}) \times 100\%.$

[0563] 3. Experimental results

[0564] The experimental results showed that sequences B207S, B1575, A1550, B1572, A1573, and A261 exhibited no off-target effects. After applying the anti-off-target design, sequences B418S-DV34P and A416-DV27P demonstrated a significant reduction in the inhibition of off-target genes without affecting the inhibition efficiency of the target gene HBV. This indicates that the anti-off-target design does not compromise the inhibitory effect of the disclosed template-modified sequences on the HBV gene but significantly suppresses off-target effects.

[0565] (i) qPCR analysis of potential off-target effects and anti-off-target efficacy of each sequence

[0566] (1) The inhibition efficiency of each sequence on the target gene and off-target genes

is shown in Table 47. Sequence A416 exhibited a clear concentration-dependent inhibition of the SLCO2B1 gene, with a maximum inhibition rate of 40.05%. Sequence B418S showed a certain concentration-dependent inhibition of the SLC41A2 gene, with a maximum inhibition rate of 24.13%. The inhibition rates of the remaining sequences (B207S, A1550, and A416) on off-target genes were all below 20%, suggesting no off-target effects. Sequences A416 and B418S were associated with a higher number of potential off-target genes, indicating that these sequences may have potential off-target effects.

[0567] Table 47: Inhibition effects of each sequence on off-target genes and the target

gene				
Unmodified sequence code	Sequence code	Gene	IC50 (nM)	Maximum inhibition rate (%)
B207S	B207S-DV29P	SEPTIN6	>30	<20
		AAK1	>30	<20
		CCSAP	>30	<20
		COX5A	>30	<20
		ABCB10	>30	<20
		IRS1	>30	<20
		ELP1	>30	<20
		NUS1	>30	<20
		SUB1	>30	<20
		AFF4	>30	<20
		NDUFA5	>30	<20
		CSNK1D	>30	<20
		EXOC1	>30	<20
		HBV	0.46	NA
A1550	B1550-DV27P	USP36	>30	<20
		CHMP7	>30	<20
		HBV	0.84	NA
A416	B416-DV27P	SLCO2B1	>30	40.05
		SLC41A2	>30	<20
		HBV	1.59	NA
B418S	B418S-DV34P	SLC41A2	>30	24.13
		HBV	0.27	NA

[0568] (2) Anti-off-target designs d7B and d67B were applied to sequences A416 and B418S. The inhibition effects of the anti-off-target designed sequences and the non-anti-off-target designed sequences on off-target genes and the target gene are shown in Table 48. Results indicate that after the anti-off-target modification with d7B, the IC50 of sequence C416-

DV27Pd7B for the off-target gene SLCO2B1 increased by 5.69-fold, and the maximum inhibition rate decreased by 9.54%. The maximum inhibition rate of sequence C418S-DV34Pd7B for the off-target gene SLC41A2 decreased by 16.91%, significantly outperforming the anti-off-target modifications d67B and GNA. Meanwhile, the IC₅₀ of sequence C418S-DV34Pd7B for the target gene HBV was 0.22 nM, which is comparable to that of the non-anti-off-target modified sequence B418S-DV34P. The maximum inhibition rates for the target gene were both around 81%, indicating that the d7B modification did not affect the inhibitory effect of sequence C418S-DV34Pd7B on the target gene.

[0569] Table 48: Inhibition effects of anti-off-target modified sequences on off-target genes and the target gene

Gene	Unmodified sequence code	Sequence code	IC ₅₀ (nM)	Maximum inhibition rate (%)
SLCO2B1	A416	B416-DV27P	23	63.83
		C416-DV27Pd67B	>150	48.72
		C416-DV27Pd7B	130.76	54.29
		C416-DV27P+	>150	28.53
SLC41A2	A416	B416-DV27P	>150	1.15
		C416-DV27Pd67B	>150	7.63
		C416-DV27Pd7B	>150	4.41
		C416-DV27P+	>150	30.64
	B418S	B418S-DV34P	>150	17.89
		C418S-DV34Pd67B	>150	16.48
		C418S-DV34Pd7B	>150	0.98
		C418S-DV34P+	>150	11.81
HBV	A416	B416-DV27P	0.79	74.67
		C416-DV27Pd67B	1.99	74.93
		C416-DV27Pd7B	3.88	70.57
		C416-DV27P+	3.29	83.93
	B418S	B418S-DV34P	0.16	81.58
		C418S-DV34Pd67B	0.24	89.56
		C418S-DV34Pd7B	0.22	81.84
		C418S-DV34P+	1.4	68.55

[0570] Example 7: Inhibition of HBV in mice by sequences modified with the templates (high dose) of the present disclosure

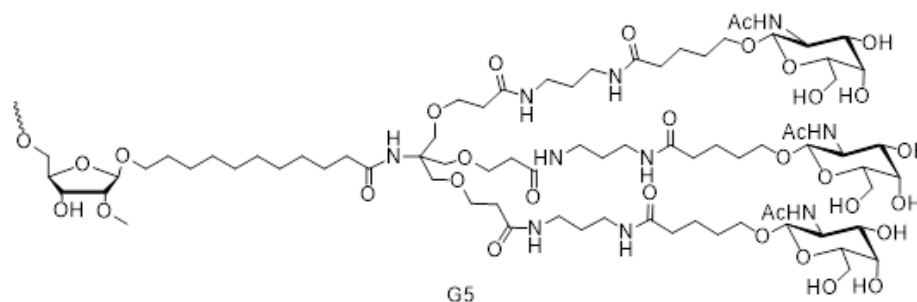
[0571] This example exemplarily selects several sequences, including unmodified sequences B418S, A416, B1550, B207S, B1575, B1572, and A1573. These sequences were modified,

for example, using only template modifications or combining template modifications with anti-off-target designs, and all were conjugated with GalNAc ligands. The in vivo efficacy of these RNAi agents at a high dose (e.g., 3 mg/kg) after repeated administration was analyzed using an AAV-HBV mouse model. This mouse model, after infection with a recombinant adeno-associated virus (AAV) carrying a replicable HBV genome, can continuously produce HBV viral particles and HBV antigens for over one year without seroconversion, replicating some immunological features of clinical chronic hepatitis B patients. Therefore, this model is also used to evaluate new immune-based therapies and antiviral treatments. In this example, the AAV-HBV mouse model was used to detect the inhibitory effects of the aforementioned sequences on serum HBsAg, HBeAg, and HBV DNA at different time points, as well as their impact on the reconstruction of adaptive immune function in mice at specific time points.

[0572] 1. Experimental materials

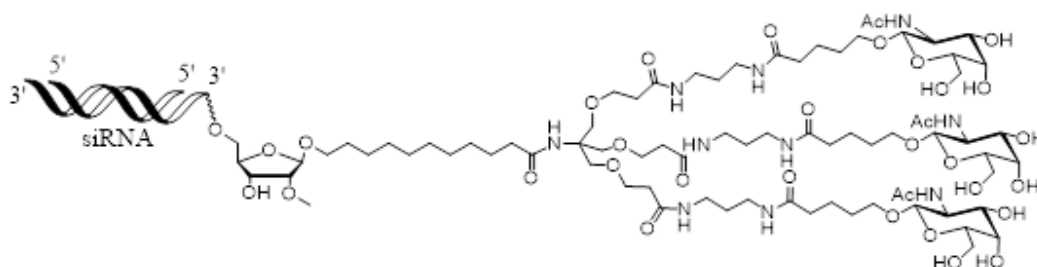
[0573] Test compounds:

[0574] The sequences listed in Table 49 include sequences with only template modifications and sequences with both template modifications and anti-off-target designs. The 3' end of the sense strand of these sequences is conjugated with the GalNAc ligand G5, with the structural formula as follows:



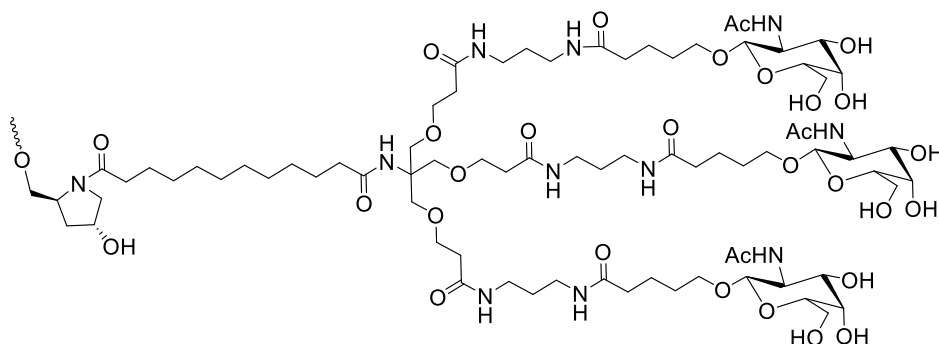
[0575] The conjugation method of the oligonucleotide with the ligand G5 refers to the preparation method described in Example 3 of the patent application CN116854754A.

[0576] The oligonucleotide and ligand G5 form the conjugate as shown below:



[0577] The specific sequences of each sequence are listed in Table 49. In the sequence codes, "G5" indicates that the sequence is conjugated with the GalNAc ligand G5, and "GL" indicates that the sequence is conjugated with the GalNAc ligand L96.

[0578] The structural formula of L96 is as follows:



[0579] Table 49: siRNA sequences used in animal experiments

Unmodified sequence code	Sequence code	Sense strand sequence 5'-3'	Sequence ID No	Unmodified sequence ID No	Antisense strand sequence 5'-3'	Sequence ID No	Unmodified sequence ID No
B207S	C207 S-DV29 PG5	Gms-Gms-Gm-Gm-Um-Uf-Um-Uf-Um-Cf-Um-Um-Gm-Um-Um-Gf-Am-Cm-Am-Am-G5	326	1	UmsEVP-Ufs-Gm-Uf-Cf-Af-Am-Cm-Am-Am-Gm-Am-Am-Af-Am-Af-Cm-Cm-Cm-Cms-Gms-Cm	179	8
B207S	C207 S-DV32 PG5	Gms-Gms-Gm-Gm-Um-Uf-Um-Uf-Um-Cf-Um-Um-Gm-Um-Um-Gf-Am-Cm-Am-Am-G5	329	1	UmsEVP-Ufs-Gm-Um-Cf-Af-Am-Cm-Am-Am-Gm-Am-Am-Af-Am-Af-Cm-Cm-Cm-Cms-Gms-Cm	181	8
A1550	C155 0-DV27 PG5	Cms-Cms-Cm-Gm-Um-Cm-Uf-Gm-Uf-Gf-Cf-Cm-Um-Um-Cm-Um-Cf-Am-Um-Cm-Um-G5	332	2	AmsEVP-Gfs-Am-Uf-Gf-Af-Gm-Am-Am-Gm-Gm-Cm-Am-Cf-Am-Gf-Am-Cm-Gm-Gm-Gms-Gms-Am	187	9
B1572	C157 2-DV27 PG5	Cms-Cms-Gm-Gm-Am-Cm-Cf-Gm-Uf-Gf-Uf-Gm-Cm-Am-Cm-Um-Uf-Cm-Gm-Cm-Um-G5	335	3	AmsEVP-Gfs-Cm-Gf-Af-Af-Gm-Um-Gm-Cm-Am-Cm-Am-Cf-Gm-Gf-Um-Cm-Cm-Gm-Gms-Cms-Am	195	10
B1575	C157 5-DV27 PG5	Gms-Ams-Cm-Cm-Gm-Um-Gf-Um-Gf-Cf-Af-Cm-Um-Um-Cm-Gm-Cf-Um-Um-Cm-Am-G5	338	4	UmsEVP-Gfs-Am-Af-Gf-Cf-Gm-Am-Am-Gm-Um-Gm-Cm-Af-Cm-Af-Cm-Gm-Gm-Um-Cms-Cms-Gm	201	11
B1575	C157 5-	Gms-Ams-Cm-Cm-Gm-Um-Gf-Um-Gf-Cm-Af-	341	4	UmsEVP-Gfs-Am-Af-Gf-Cf-Gm-Am-Am-Gm-Um-Gm-	201	11

	DV29 PG5	Cm-Um-Um-Cm-Gm-Cf- Um-Um-Cm-Am-G5			Cm-Af-Cm-Af-Cm-Gm-Gm- Um-Cms-Cms-Gm		
A1573	C157 3- DV29 PG5	Cms-Gms-Gm-Am-Cm- Cm-Gf-Um-Gf-Um-Gf- Cm-Am-Cm-Um-Um-Cf- Gm-Cm-Um-Um-G5	344	5	AmsEVP-Afs-Gm-Cf-Gf-Af- Am-Gm-Um-Gm-Cm-Am- Cm-Af-Cm-Gf-Gm-Um-Cm- Cm-Gms-Gms-Cm	207	12
A416	C416- DV27 Pd7B G5	Ums-Gms-Cm-Um-Gm- Cm-Uf-Am-Uf-Gf-Cf-Cm- Um-Cm-dA-Um-Cf-Um- Um-Cm-Um-G5	347	7	AmsEVP-Gfs-Am-Af-Gf-Af- dT-Gm-Am-Gm-Gm-Cm- Am-Uf-Am-Gf-Cm-Am-Gm- Cm-Ams-Gms-Gm	318	14
B418S	C418 S- DV34 Pd7B G5	Cms-Ums-Gm-Cm-Um- Af-Um-Gf-Cm-Cf-Um- Cm-Am-dT-Cm-Uf-Um- Cm-Um-Um-G5	350	6	AmsEVP-Afs-Gf-Am-Am- Gf-dA-Um-Gm-Am-Gm- Gm-Cm-Af-Um-Af-Gm-Cm- Am-Gms-Cms-Am	321	13
APC	VIR- 2218- GL	Gms-Ums-Gm-Um-Gf- Cm-Af-Cf-Uf-Um-Cm- Gm-Cm-Um-Um-Cm-Am- Cm-Am-GL	353	45	Ums-Gfs-Um-Gm-Am- A(gna)-Gm-Cf-Gf-Am-Am- Gm-Um-Gf-Cm-Af-Cm-Am- Cms-Ums-Um	294	79
ANC	CNC- G5	Gms-Gms-Um-Cm-Um- Cm-Uf-Um-Cf-Um-Af- Um-Cm-Gm-Am-Um-Cm- Um-Um-Am-Um-G5	354	46	AmsEVP-Ufs-Am-Af-Gf-Af- Um-Cm-Gm-Am-Um-Am- Gm-Af-Am-Gf-Am-Gm- Am-Cm-Cms-Cms-Am	295	80

[0580] Preparation of test compounds:

[0581] Solvent: PBS buffer

[0582] Preparation conditions: Sterile environment

[0583] Labeling Method: Prepared dosing formulations are labeled with tags indicating the project number, name, concentration, quantity, preparation date, preparer, and storage conditions on the outer packaging.

[0584] Storage conditions: Prepared immediately before use; remaining samples are stored at -80°C.

[0585] Experimental animal information:

[0586] Species/Strain: AAV-HBV mice

[0587] Grade: SPF (Specific Pathogen-Free)

[0588] Gender: Male

[0589] Quantity: 100

[0590] Age: 7 weeks

[0591] Weight: 19–24 g

[0592] Source: Guangdong Zhiyuan Biomedical Technology Co., Ltd.

[0593] Production license number: SCXK (Yue) 2021-0057

[0594] Institutional Animal Care and Use Committee (IACUC):

[0595] After receipt, the experimental animals were feeded at GuangZhou Jennio Biotech Co.,Ltd., with the license number: JENNIO (Jinbin) 2019-0002. This project has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of GuangZhou Jennio Biotech Co.,Ltd., with the IACUC approval number JENNIO-IACUC-2024-A005. The experimental procedures were strictly conducted in accordance with IACUC requirements to ensure animal welfare.

[0596] Feeding and management:

[0597] Feeding conditions: After receipt, the experimental animals were housed at Guangzhou Jennio Biotech Co., Ltd. The animals were feeded in cages with dimensions of length × width × height = 29.0 cm × 18.5 cm × 13.0 cm. The environmental conditions were set to a temperature range of 20–26°C and a humidity range of 40%–70%, with automatic lighting on a 12-hour light/dark cycle.

[0598] The feeding environment conditions adhered to the National Standard of the People's Republic of China GB14925-2010.

[0599] Animals were provided with ad libitum access to food and water. The feed consisted of irradiated sterilized maintenance diet for experimental mice, supplied by Jiangsu Collaborative Medical Biotechnology Co., Ltd., with the production license number Su Feed Certificate (2019) 01008. The nutritional composition of the feed was tested in accordance with the National Standard of the People's Republic of China GB14924.3-2010, and the pollutant content was tested in accordance with the National Standard of the People's Republic of China GB14924.2-2001. The feed supplier provided a test report for each batch. Drinking water was reverse osmosis water, provided in water bottles. The drinking water was tested in accordance with the National Standard of the People's Republic of China GB5750-2006 and sent to a third-party testing agency for annual testing.

[0600] The animal bedding consisted of corncob bedding, supplied by Guangzhou Saibainuo Biotechnology Co., Ltd., with the production license number SCXK (Jing) 2019-0004. The

pollutant content of the bedding was tested in accordance with the National Standard of the People's Republic of China GB14924.2-2001, and the bedding supplier provided a test report for each batch.

[0601] Animal cages and bedding were replaced at least once a week. All cages and bedding were sterilized using a pulse vacuum sterilizer before being introduced into the barrier environment. Animal cage racks were cleaned and disinfected at least once a week.

[0602] The animal feeding observation rooms were cleaned and disinfected daily, including shelves, floors, tables, and other surfaces.

[0603] The disinfectants used in the barrier environment include: 6.67% benzalkonium bromide solution, 0.5% 84 disinfectant, 75% disinfectant, and 0.08% Bestaquam. These four disinfectants are to be used in rotation and must not be mixed.

[0604] 2. Experimental methods

[0605] 2.1 AAV-HBV mouse model preparation

[0606] Definition of experimental timeline: The day of administration of the vehicle or test substance to the animals is defined as Day 0.

[0607] One hundred SPF-grade C57BL/6 male mice were acclimatized in a barrier facility for 7 days, with daily observations to confirm their health and absence of abnormalities before model preparation. The mice were injected via the tail vein with rAAV8-1.3HBV (produced by Guangzhou PackGene Biotechnology Co.,Ltd.; product name: AAV8HBV-D,ayw, batch number: HBV101-6) at a dose of 1×10^{11} GC/100 μ L per mouse. After model preparation, blood was collected from the animals at weeks 5 and 6 (D-14 and D-7), followed by centrifugation and plasma collection to measure HBV DNA, HBsAg, and HBeAg levels.

[0608] 2.2 Animal grouping and administration

[0609] Definition of experimental timeline: The day of administration of the vehicle or test substance to the animals is defined as Day 0 (D0).

[0610] Grouping was performed based on the indicators at week 6 (D-7) of model preparation. Seventy-two successfully modeled animals were selected and randomly divided into 12 groups of 6 mice each according to HBsAg levels. The average HBsAg levels were ensured to be comparable across groups, with no statistically significant differences in HBV DNA and HBeAg levels between groups. Administration began at week 7 (D0) of model preparation,

with details on grouping and administration shown in Table 50.

[0611] Table 50: Group settings and administration details

Group	Administration regimen	Administration route	Number of animals
siRNA (11 groups)	3 mg/kg, administered on D0, D7, and D14	s.c.	6 mice/group × 11 groups
Vehicle control (1 group)	PBS, administered once on D0, D7, and D14	s.c.	6 mice/group × 1 group

[0612] Note: s.c. represents subcutaneous injection.

[0613] 2.3 Secondary challenge experiment

[0614] On D60 of administration, 3 mice from the C207S-DV32PG5 group were selected for hydrodynamic tail vein injection with 8 µg of pAAV-HBV 1.2 plasmid (Fenghui Biotech) at an injection volume of 100 µL/g for the challenge. The remaining 3 mice were left untreated. Additionally, 3 mice from a blank control group were subjected to the same challenge procedure as a control.

[0615] 2.4 Observation and indicator monitoring

[0616] (1) General observation

[0617] During both the model preparation and experimental periods, animals were observed daily, and records were maintained. Observations included: mortality, moribund state, feed and water intake, injuries, feces, appearance and coat condition, mental state, and activity levels.

[0618] (2) Body weight

[0619] Acclimatization period: Body weights were recorded upon animal receipt and on the final day of the acclimatization period.

[0620] Experimental period: Body weights were recorded weekly during the experimental period. If administration or blood collection was scheduled on the same day, weights were measured prior to the procedure or before euthanasia.

[0621] (3) Measurement of serum HBsAg, HBeAg, HBV DNA, HBsAb, and ALT levels

[0622] Blood collection (200 µL) was performed via the retro-orbital sinus at Week 5 (D-14) and Week 6 (D-7) post-modeling, as well as before administration (D 0) and weekly thereafter. Blood was collected into EDTA-K2 anticoagulant tubes and centrifuged at 1000 × g for 10 minutes to obtain plasma. For mice subjected to the secondary challenge, blood collection

(200 μ L) was performed via the retro-orbital sinus on D60 (challenge day), followed by additional collections on D63, D67, D74, and D81. Plasma was obtained as described above. For analysis, 20 μ L of plasma was diluted with 980 μ L of PBS, vortexed, and used for measuring HBsAg, HBV DNA, HBeAg, and ALT levels. Additionally, 15 μ L of plasma was diluted with 210 μ L of PBS, vortexed, and used for measuring HBsAb levels. All processed samples were analyzed by Guangzhou Huayin Medical Testing Center Co., Ltd. Remaining plasma samples were stored at -80°C for future use.

[0623] 3. Experimental results

[0624] The experimental results demonstrated that, compared to the vehicle control group and the negative control group, the sequences B207S-DV32P, A1550-DV27P, B1572-DV27P, B1575-DV27P, B207S-DV29P, A1573-DV29P, B1575-DV29P, B418S-DV34Pd7B, and A416-DV27Pd7B, when conjugated with G5, were effectively targeted to the liver and exhibited sustained and significant suppression of plasma HBsAg, HBeAg, and HBV DNA levels.

[0625] Among these, the sequences C207S-DV32PG5, C207S-DV29PG5, and C418S-DV34Pd7BG5 demonstrated superior efficacy in reducing HBsAg, HBV DNA, and HBeAg levels, significantly outperforming the positive control sequence VIR-2218-GL. Furthermore, the sequences C207S-DV32PG5 and C207S-DV29PG5 achieved complete seroclearance of HBsAg and HBV DNA in all mice within their respective groups, accompanied by the generation of high levels of antibodies. In the C418S-DV34Pd7BG5 group, 3 out of 6 mice also achieved HBsAg seroclearance, along with the production of high antibody levels.

[0626] The results of the secondary challenge experiment showed that the blank control group (mice not previously infected with HBV before D56) exhibited detectable levels of HBsAg, HBeAg, and HBV DNA post-challenge, confirming the effectiveness of the challenge method. In the C207S-DV32PG5 challenge group, HBV DNA levels initially increased post-challenge but subsequently declined to below the detection limit. Concurrently, serum HBsAb levels significantly increased, while HBsAg remained below the detection limit. These results confirm the effectiveness of the challenge and demonstrate that mice treated with this small nucleic acid sequence were protected against reinfection with HBV. This evidence proves that HBV-infected mice treated with this small nucleic acid sequence are protected against

subsequent HBV viral attacks.

[0627] (i) Temporal changes in HBsAg, HBeAg, HBV DNA, HBsAb, and ALT levels in mice across sequence groups

[0628] The temporal changes in HBsAg, HBeAg, HBV DNA, HBsAb, ALT, and body weight levels in mice across the groups are shown in Tables 51-59 and FIGs 1A-1F. The data indicate that the sequences C207S-DV32PG5, C207S-DV29PG5, and C418S-DV34Pd7BG5 exhibited superior efficacy in reducing HBsAg, HBV DNA, and HBeAg levels, significantly outperforming the positive control sequence VIR-2218-GL. Furthermore, the sequences C207S-DV32PG5 and C207S-DV29PG5 achieved complete seroclearance of HBsAg and HBV DNA in all mice within their respective groups, accompanied by the generation of high levels of antibodies. Specifically, the C207S-DV32PG5 group achieved complete seroclearance of HBsAg and HBV DNA in all mice by Day 14 and Day 21 post-administration, respectively, and this effect persisted until the end of the experiment. These results suggest that the mice achieved functional cure. In the C418S-DV34Pd7BG5 group, 3 out of 6 mice also achieved HBsAg seroclearance, along with the production of high antibody levels. These data collectively demonstrate the superior therapeutic efficacy of the sequences C207S-DV32PG5, C207S-DV29PG5, and C418S-DV34Pd7BG5.

[0629] Table 51: Changes in plasma HBsAg levels in mice across sequence groups

Unmodified sequence code	Sequence code	Log10 HBsAg (IU/mL)										
		D-7	D0	D7	D14	D21	D28	D35	D42	D49	D56	D63
	PBS	3.27 ±0.6 0	3.16 ±0.5 0	3.71± 0.51	3.64± 0.34	3.85± 0.35	4.08± 0.40	4.12± 0.36	3.96± 0.48	4.17± 0.35	4.17± 0.44	4.14± 0.51
APC	VIR-2218-GL	3.49 ±0.1 6	3.09 ±0.3 8	1.61± 0.17* *	1.01± 0.19* *	0.74± 0.28* *	1.15± 0.23* *	1.26± 0.22* *	1.54± 0.45* *	1.63± 0.25	1.75± 0.29	2.29± 0.44
A1550	C1550-DV27PG5	3.49 ±0.1 5	3.48 ±0.1 8	2.83± 0.21	2.38± 0.40	2.18± 0.47	2.28± 0.48	2.51± 0.65	2.63± 0.52	-	-	-
B1572	C1572-DV27PG5	3.50 ±0.1 3	3.44 ±0.3 6	2.76± 0.54	2.39± 0.58	2.29± 0.52	2.49± 0.50	2.58± 0.77	2.65± 0.73	-	-	-

A1573	C1573-DV29PG5	3.50±0.13	3.44±0.30	2.98±0.56	2.64±0.44	2.67±0.38	2.62±0.35	2.76±0.39	2.67±0.51	-	-	-
B1575	C1575-DV27PG5	3.49±0.15	3.45±0.24	1.62±0.51*	1.24±0.56*	1.22±0.50*	1.47±0.61*	1.71±0.66*	1.73±0.68*	2.12±0.43	2.07±0.45	1.97±0.74
B1575	C1575-DV29PG5	3.50±0.12	3.40±0.18	2.31±0.29	1.76±0.34	1.93±0.44	2.23±0.36	2.29±0.31	2.48±0.29	-	-	-
B207S	C207S-DV29PG5	3.50±0.11	3.69±0.27	1.88±0.32*	0.43±0.08*	0.41±0.03*	0.43±0.08*	0.45±0.12*	0.45±0.12*	0.40±0.00*	0.41±0.03*	0.40±0.00**
B207S	C207S-DV32PG5	3.50±0.13	3.35±0.26	1.29±0.53*	0.40±0.00*	0.40±0.00*	0.40±0.00*	0.40±0.00*	0.40±0.00*	0.40±0.00*	0.40±0.00*	/
A416	C416-DV27Pd7BG5	3.47±0.20	3.47±0.16	1.90±0.30*	1.52±0.31	1.44±0.34	1.79±0.31	2.08±0.27	2.41±0.13	-	-	-
B418S	C418S-DV34Pd7BG5	3.47±0.23	3.41±0.26	1.27±0.50*	0.60±0.40*	0.51±0.27*	0.53±0.31*	0.49±0.22*	0.61±0.45*	0.59±0.35*	0.70±0.51*	0.95±0.69**
ANC	CNC-G5	3.47±0.22	3.43±0.23	3.83±0.21	3.66±0.29	3.82±0.32	3.88±0.20	4.02±0.30	4.17±0.30	4.02±0.35	4.04±0.35	-

[0630] Notes: “-” indicates that testing was discontinued. “/” indicates that the C207S-DV32PG5 group was selected for the challenge experiment on D56. Compared to the PBS group: * indicates $P < 0.05$; ** indicates $P < 0.01$. Compared to the positive control group (VIR-2218-GL): # indicates $P < 0.05$; ## indicates $P < 0.01$.

[0631] Table 52: Changes in plasma HBV DNA levels in mice across sequence groups

Unmodified sequence code	Sequence code	Log10 HBV DNA (IU/mL)										
		D-7	D0	D7	D14	D21	D28	D35	D42	D49	D56	D63
	PBS	6.76±0.75	6.25±0.61	6.98±0.54	7.22±0.35	7.40±0.30	7.55±0.17	7.79±0.24	7.64±0.11	7.84±0.16	8.01±0.28	7.78±0.90
APC	VIR-2218-GL	6.93±0.30	6.47±0.63	5.22±0.36*	4.66±0.33*	4.75±0.53*	4.72±0.69*	4.64±0.76*	5.45±0.64*	5.54±0.48	5.69±0.69	6.44±0.69
A1550	C1550-DV27PG5	7.01±0.47	7.12±0.33	6.92±0.41	6.20±0.67	5.94±0.80	6.04±0.99	6.21±1.00	6.79±0.69	-	-	-

B1572	C1572-DV27PG5	6.83±0.50	6.30±0.78	6.10±1.16	5.86±1.03	5.90±0.92	5.82±1.19	6.09±1.31	6.69±1.16	-	-	-
A1573	C1573-DV29PG5	7.14±0.22	6.63±0.78	6.56±0.74	6.43±0.69	6.37±0.38	6.37±0.32	6.59±0.4	6.63±0.67	-	-	-
B1575	C1575-DV27PG5	6.98±0.39	6.81±0.50	5.53±0.44*	5.18±0.49	5.08±0.51*	5.34±0.30	5.73±0.42*	5.93±0.33	6.19±0.40	6.39±0.71	6.04±0.81
B1575	C1575-DV29PG5	6.94±0.29	6.65±0.27	5.96±0.53*	5.59±0.55	5.59±0.75	5.99±0.70	5.96±0.43	6.46±0.61	-	-	-
B207S	C207S-DV29PG5	6.96±0.34	7.14±0.46	5.61±0.56*	3.76±0.19*	3.54±0.00*	3.54±0.00*	3.54±0.00*	3.54±0.00*	3.54±0.00*	3.54±0.00*	3.54±0.00*
B207S	C207S-DV32PG5	6.96±0.39	6.65±0.50	5.92±0.38*	3.84±0.45*	3.54±0.00*	3.54±0.00*	3.54±0.00*	3.54±0.00*	3.54±0.00*	3.54±0.00*	/
A416	C416-DV27Pd7BG5	6.99±0.40	6.90±0.40	5.94±0.51*	5.16±0.39	5.14±0.59*	5.21±0.49*	6.10±0.39	6.73±0.42	-	-	-
B418S	C418S-DV34Pd7BG5	6.94±0.52	6.95±0.50	5.47±0.38*	4.38±0.52*	4.23±0.24*	3.72±0.44*	3.70±0.32*	4.04±0.59*	4.03±0.52*	4.39±0.41*	4.78±0.74*
ANC	CNC-G5	6.76±0.89	6.65±0.90	7.14±0.63	7.33±0.56	7.41±0.54	7.53±0.44	7.48±0.51	7.79±0.67	7.66±0.47	7.77±0.70	/

[0632] Notes: “-” indicates that testing was discontinued. “/” indicates that the C207S-DV32PG5 group was selected for the challenge experiment on D56. Compared to the PBS group: * indicates $P < 0.05$; ** indicates $P < 0.01$. Compared to the positive control group (VIR-2218-GL): # indicates $P < 0.05$; ## indicates $P < 0.01$.

[0633] Table 53: Changes in plasma HBeAg levels in mice across sequence groups

Unmodified sequence code	Sequence code	Log10 HBeAg (S/CO)										
		D-7	D0	D7	D14	D21	D28	D35	D42	D49	D56	D63
/	PBS	3.76±0.06	3.76±0.06	3.40±0.17	3.72±0.05	3.63±0.08	3.70±0.06	3.74±0.05	3.75±0.06	3.75±0.04	3.66±0.06	3.66±0.10
APC	VIR-2218-GL	3.80±0.08	3.76±0.05	2.80±0.28*	2.96±0.27*	2.79±0.25*	2.90±0.24*	3.09±0.26*	3.18±0.22*	3.27±0.20*	3.24±0.22*	3.36±0.20

A1550	C1550-DV27PG5	3.77±0.06	3.72±0.09	3.00±0.31	3.26±0.19	3.09±0.18*	3.22±0.19	3.34±0.18	3.37±0.17	-	-	-
B1572	C1572-DV27PG5	3.74±0.08	3.75±0.06	3.25±0.31	3.33±0.28	3.12±0.26*	3.25±0.26	3.33±0.26	3.39±0.27	-	-	-
A1573	C1573-DV29PG5	3.78±0.04	3.75±0.09	3.33±0.18	3.51±0.07	3.34±0.07*	3.46±0.05	3.56±0.03	3.52±0.06	-	-	-
B1575	C1575-DV27PG5	3.77±0.05	3.71±0.05	2.69±0.33*	2.78±0.26*	2.68±0.14*	2.87±0.17*	3.04±0.17*	3.14±0.14*	3.20±0.10*	3.24±0.12*	3.30±0.07
B1575	C1575-DV29PG5	3.76±0.07	3.73±0.04	2.95±0.23	3.07±0.26*	2.98±0.24*	3.17±0.20	3.25±0.17	3.32±0.13	-	-	-
B207S	C207S-DV29PG5	3.75±0.03	3.71±0.09	2.99±0.16	3.02±0.07*	2.75±0.12*	2.76±0.10*	2.73±0.08*	2.73±0.09*	2.64±0.13*	2.59±0.11*	2.61±0.14*
B207S	C207S-DV32PG5	3.75±0.03	3.75±0.05	2.79±0.23*	2.83±0.11*	2.50±0.19*	2.54±0.11*	2.57±0.09*	2.61±0.10*	2.56±0.09*	2.47±0.09*	/
A416	C416-DV27Pd7BG5	3.75±0.05	3.77±0.06	3.25±0.14	3.49±0.09	3.33±0.06*	3.51±0.06	3.61±0.06	3.67±0.04	-	-	-
B418S	C418S-DV34Pd7BG5	3.70±0.10	3.70±0.09	3.17±0.18	3.29±0.11	3.09±0.15*	2.94±0.22*	3.02±0.22*	3.01±0.23*	2.94±0.24*	2.89±0.24*	2.90±0.18*
ANC	CNC-G5	3.76±0.07	3.71±0.07	3.32±0.14	3.66±0.06	3.70±0.06	3.69±0.05	3.76±0.09	3.74±0.08	3.71±0.08	3.64±0.06	/

[0634] Notes: “-” indicates that testing was discontinued. “/” indicates that the C207S-DV32PG5 group was selected for the challenge experiment on D56. Compared to the PBS group: * indicates $P < 0.05$; ** indicates $P < 0.01$. Compared to the positive control group (VIR-2218-GL): # indicates $P < 0.05$; ## indicates $P < 0.01$.

[0635] **Table 54: Changes in the number of mice with HBsAg clearance across sequence groups**

Unmodified sequence code	Sequence code	Number of mice with HBsAg clearance								
		D7	D14	D21	D28	D35	D42	D49	D56	D63
B418S	C418S-DV34Pd7BG5	0/6	4/6	5/6	5/6	5/6	4/6	4/6	4/6	3/6

B207S	C207S-DV32PG5	0/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
B1575	C1575-DV27PG5	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
B207S	C207S-DV29PG5	0/6	5/6	5/6	5/6	5/6	5/6	6/6	5/6	6/6
APC	VIR-2218-GL	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
A416	C416-DV27Pd7BG5	0/6	0/6	0/6	0/6	0/6	0/6	NA	NA	NA
B1575	C1575-DV29PG5	0/6	0/6	0/6	0/6	0/6	0/6	NA	NA	NA
B1572	C1572-DV27PG5	0/6	0/6	0/6	0/6	0/6	0/6	NA	NA	NA
A1550	C1550-DV27PG5	0/6	0/6	0/6	0/6	0/6	0/6	NA	NA	NA
A1573	C1573-DV29PG5	0/6	0/6	0/6	0/6	0/6	0/6	NA	NA	NA
ANC	CNC-G5	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
	PBS	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6

[0636] Table 55: Changes in the number of mice with HBV DNA clearance across sequence groups

Unmodified sequence code	Sequence code	Number of mice with HBV DNA clearance								
		D7	D14	D21	D28	D35	D42	D49	D56	D63
B418S	C418S-DV34Pd7BG5	0/6	0/6	0/6	5/6	4/6	2/6	0/6	0/6	0/6
B207S	C207S-DV32PG5	0/6	3/6	6/6	6/6	6/6	6/6	6/6	6/6	2/6
B1575	C1575-DV27PG5	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
B207S	C207S-DV29PG5	0/6	1/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
APC	VIR-2218-GL	0/6	0/6	0/6	1/6	1/6	0/6	0/6	0/6	0/6
A416	C416-DV27Pd7BG5	0/6	0/6	0/6	0/6	0/6	0/6	NA	NA	NA
B1575	C1575-DV29PG5	0/6	0/6	0/6	0/6	0/6	0/6	NA	NA	NA
B1572	C1572-DV27PG5	0/6	0/6	0/6	1/6	0/6	0/6	NA	NA	NA

A1550	C1550-DV27PG5	0/6	0/6	0/6	0/6	0/6	0/6	0/6	NA	NA	NA
A1573	C1573-DV29PG5	0/6	0/6	0/6	0/6	0/6	0/6	0/6	NA	NA	NA
ANC	CNC-G5	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
	PBS	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6

[0637] Table 56: Changes in the number of mice producing antibodies across sequence groups

Unmodified sequence code	Sequence code	Number of mice producing antibodies									
		D7	D14	D21	D28	D35	D42	D49	D56	D63	
B418S	C418S-DV34Pd7BG5	2/6	4/6	4/6	4/6	6/6	6/6	6/6	6/6	4/6	
B207S	C207S-DV32PG5	2/6	2/6	3/6	3/6	4/6	4/6	4/6	5/6	5/6	
B1575	C1575-DV27PG5	0/6	1/6	2/6	0/6	0/6	2/6	2/6	2/6	2/6	
B207S	C207S-DV29PG5	1/6	4/6	1/6	2/6	3/6	5/6	5/6	4/6	5/6	
APC	VIR-2218-GL	2/6	3/6	3/6	3/6	3/6	3/6	2/6	2/6	1/6	
A416	C416-DV27Pd7BG5	2/6	2/6	3/6	2/6	0/6	0/6	NA	NA	NA	
B1575	C1575-DV29PG5	0/6	0/6	0/6	0/6	0/6	0/6	NA	NA	NA	
B1572	C1572-DV27PG5	0/6	0/6	0/6	0/6	0/6	0/6	NA	NA	NA	
A1550	C1550-DV27PG5	0/6	0/6	0/6	0/6	0/6	0/6	NA	NA	NA	
A1573	C1573-DV29PG5	0/6	0/6	0/6	0/6	0/6	1/6	NA	NA	NA	
ANC	CNC-G5	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	
	PBS	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	

[0638] Table 57: Changes in plasma HBsAb levels in mice across sequence groups

Unmodified sequence code	Sequence code	HBsAb (mIU/mL)									
		D7	D14	D21	D28	D35	D42	D49	D56	D63	

B418S	C418S-DV34Pd7BG5	35	117	257	180	145	191	311	293	363
B207S	C207S-DV32PG5	52	88	69	65	84	111	237	383	597
B1575	C1575-DV27PG5	30	30	34	30	30	33	34	49	37
B207S	C207S-DV29PG5	37	39	31	31	64	119	179	172	264
APC	VIR-2218-GL	31	92	226	306	362	203	161	142	97
A416	C416-DV27Pd7BG5	33	36	39	34	30	30	NA	NA	NA
B1575	C1575-DV29PG5	30	30	30	30	30	30	NA	NA	NA
B1572	C1572-DV27PG5	30	30	30	30	30	30	NA	NA	NA
A1550	C1550-DV27PG5	30	30	30	30	30	30	NA	NA	NA
A1573	C1573-DV29PG5	30	30	30	30	30	31	NA	NA	NA
ANC	CNC-G5	30	30	30	30	30	30	30	30	30
	PBS	30	30	30	30	30	30	30	30	30

[0639] Table 58: Changes in plasma ALT levels in mice across sequence groups

Unmodified sequence code	Sequence code	ALT (U/L)								
		D7	D14	D21	D28	D35	D42	D49	D56	D63
B418S	C418S-DV34Pd7BG5	75	83	138	75	75	80	75	80	75
B207S	C207S-DV32PG5	75	78	75	78	75	78	75	75	95
B1575	C1575-DV27PG5	75	75	75	75	75	75	75	75	78
B207S	C207S-DV29PG5	75	75	75	98	75	78	223	75	75
APC	VIR-2218-GL	75	75	85	75	75	75	98	105	103
A416	C416-DV27Pd7BG5	75	75	75	75	75	75	NA	NA	NA
B1575	C1575-DV29PG5	75	75	75	75	75	75	NA	NA	NA
B1572	C1572-DV27PG5	75	75	75	83	75	75	NA	NA	NA
A1550	C1550-DV27PG5	75	80	88	83	75	75	NA	NA	NA
A1573	C1573-DV29PG5	75	75	75	75	75	75	NA	NA	NA
ANC	CNC-G5	75	75	75	75	75	75	75	75	83
	PBS	75	75	75	75	75	75	75	75	78

[0640] Table 59: Changes in body weight of mice across sequence groups

Unmodified sequence code	Sequence code	Body weight (g)								
		D7	D14	D21	D28	D35	D42	D49	D56	D63
B418S	C418S-DV34Pd7BG5	28.9	29.2	28.8	28.8	29.1	29.9	30.3	30.5	29.8
B207S	C207S-DV32PG5	29.2	29.7	29.9	30.0	30.2	30.8	31.0	31.2	31.2
B1575	C1575-DV27PG5	27.7	28.3	28.2	28.4	28.7	28.6	29.0	29.0	28.6

B207S	C207S-DV29PG5	27. 4	27. 6	27. 7	27. 6	27. 7	28. 1	28. 3	28. 5	28. 1
APC	VIR-2218-GL	28. 4	28. 9	29. 0	28. 6	29. 4	29. 6	30. 0	30. 2	29. 9
A416	C416- DV27Pd7BG5	29. 7	30. 6	30. 0	30. 0	29. 8	30. 8	NA	NA	NA
B1575	C1575-DV29PG5	29. 4	30. 0	30. 0	29. 4	29. 8	29. 8	NA	NA	NA
B1572	C1572-DV27PG5	29. 6	29. 9	30. 2	30. 1	29. 2	29. 2	NA	NA	NA
A1550	C1550-DV27PG5	28. 1	28. 7	28. 5	29. 0	29. 2	29. 2	NA	NA	NA
A1573	C1573-DV29PG5	27. 5	27. 6	27. 3	27. 3	27. 0	27. 6	NA	NA	NA
ANC	CNC-G5	28. 4	29. 0	29. 2	28. 6	28. 9	29. 4	29. 5	29. 7	29. 8
	PBS	29. 2	29. 8	30. 2	30. 0	30. 2	30. 2	31. 0	32. 3	32. 4

[0641] (ii) Protective effect of sequence C207S-DV32PG5 against secondary challenge in mice

[0642] The mice in the C207S-DV32PG5 group were subjected to hydrodynamic tail vein injection of HBV plasmid on D60 to simulate secondary HBV infection. As shown in FIGs 2A-2F, the blank control group (mice not previously infected with HBV before D56) exhibited detectable levels of HBsAg, HBeAg, and HBV DNA after the hydrodynamic injection of the HBV plasmid, confirming the effectiveness of the challenge method. In the C207S-DV32PG5 challenge group, HBV DNA levels initially increased post-challenge but subsequently declined to below the detection limit. Concurrently, serum HBsAb levels significantly increased, while HBsAg remained below the detection limit. These results confirm the effectiveness of the challenge and demonstrate that mice treated with this small nucleic acid sequence were protected against reinfection with HBV.

[0643] Example 8: Inhibition of HBV in mice by sequences modified with the templates (low dose) of the present disclosure

[0644] This example exemplarily selects several sequences, including sequences C207S-DV32PG5, C207S-DV29PG5, C418S-DV34Pd7BG5, and C1575-DV27PG5 from Example 7, as shown in Table 49, to analyze the in vivo efficacy of these RNAi agents at a low dose (e.g.,

0.5 mg/kg) following multiple administrations in an AAV-HBV mouse model.

[0645] 1. Experimental materials

[0646] Sequences C207S-DV32PG5, C207S-DV29PG5, C418S-DV34Pd7BG5, and C1575-DV27PG5 from Example 7 and VIR-2218-GL are shown in Table 49. And Entecavir (ETV).

[0647] 2. Experimental methods

[0648] The experimental method is the same as Example 7, with details on grouping and administration as shown in Table 60.

[0649] Table 60: Group settings and administration details

Group	Administration regimen	Administration route	Number of animals
siRNA (6 groups)	0.5 mg/kg, administered on D0, D7, and D14	s.c.	6 mice/group × 6 group
ETV (1 group)	0.1 mg/kg, daily	i.g.	6 mice/group × 1 group
Vehicle control (1 group)	PBS, administered once on D0, D7, and D14	s.c.	6 mice/group × 1 group

[0650] Note: s.c. represents subcutaneous injection; i.g. represents intragastric administration.

[0651] 3. Experimental results

[0652] The changes in HBsAg, HBeAg, HBV DNA, HBsAb, and ALT levels in mice from each group over time are shown in Tables 61-68 and FIGs 3A-3F. The experimental results indicate that the sequences C207S-DV32PG5 and C207S-DV29PG5 significantly outperformed the positive control sequence VIR-2218-GL in inhibiting HBsAg, HBV DNA, and HBeAg. Among them, C207S-DV32PG5 demonstrated the most effective results, reducing HBsAg by approximately 2.9 log₁₀ by day 56, which was about 1.4 log₁₀ higher than that of C207S-DV29PG5. Additionally, by day 56, HBsAg became undetectable in 3 mice, and 4 mice developed antibodies in the C207S-DV32PG5 group, while no mice in the C207S-DV29PG5 group showed HBsAg clearance. These data further confirm the superior therapeutic efficacy of the sequence C207S-DV32PG5.

[0653] Table 61: Changes in plasma HBsAg levels in mice across sequence groups

Unmodified sequence code	Sequence code	Log ₁₀ HBsAg (IU/mL)									
		D-7	D0	D7	D14	D21	D28	D35	D42	D49	D56

/	PBS	3.36 ±0.3 0	3.46 ±0.2 7	3.43± 0.36	3.61± 0.41	3.69± 0.59	3.81± 0.68	3.81± 0.70	3.86± 0.67	3.92± 0.65	4.10± 0.62
/	ETV	3.36 ±0.2 6	3.47 ±0.3 4	3.50± 0.33	3.54± 0.25	3.74± 0.14	3.94± 0.27	3.96± 0.39	4.04± 0.40	4.22± 0.33	4.20± 0.30
APC	VIR-2218- GL	3.33 ±0.3 3	3.60 ±0.2 4	2.47± 0.27* *	2.26± 0.45*	2.36± 0.42* *	2.62± 0.28	2.88± 0.34	3.18± 0.29	3.46± 0.47	3.59± 0.30
B157S	C157S- DV27PG5	3.34 ±0.3 4	3.40 ±0.5 2	2.96± 0.49	2.69± 0.57	2.80± 0.62*	2.95± 0.52	3.21± 0.65	3.23± 0.92	3.46± 0.68	3.64± 0.66
B207S	C207S- DV29PG5	3.33 ±0.3 3	3.51 ±0.3 0	2.70± 0.22* *	2.36± 0.29*	2.00± 0.33* *	1.88± 0.32	1.77± 0.31*	1.67± 0.27*	1.77± 0.29*	1.94± 0.40
B207S	C207S- DV32PG5	3.33 ±0.3 2	3.55 ±0.3 4	2.37± 0.43* *	1.82± 0.34* *	1.22± 0.41* *	0.90± 0.47* *	0.82± 0.44* *	0.79± 0.67* *	0.75± 0.41* *	0.63± 0.37* *
B418S	C418S- DV34Pd7B G5	3.33 ±0.3 3	3.40 ±0.3 2	2.68± 0.40* *	2.43± 0.66*	2.56± 0.69* *	2.76± 0.77	3.06± 0.63	3.30± 0.65	3.65± 0.75	3.93± 0.61

[0654] Note: Compared to the PBS group: * indicates $P < 0.05$; ** indicates $P < 0.01$.

[0655] Table 62: Changes in plasma HBV DNA levels in mice across sequence groups

Unmodified sequence code	Sequence code	Log10 HBV DNA (IU/mL)									
		D-7	D0	D7	D14	D21	D28	D35	D42	D49	D56
/	PBS	6.97 ±0.2 8	7.22 ±0.4 1	7.20± 0.49	7.54± 0.72	7.55± 0.63	7.65± 0.57	7.65± 0.46	7.72± 0.56	7.96± 0.35	8.05± 0.57
/	ETV	6.65 ±0.5 8	6.91 ±0.5 8	4.49± 0.54* *	3.85± 0.24* *	3.89± 0.31* *	3.77± 0.37* *	3.74± 0.25* *	3.63± 0.08* *	3.97± 0.13* *	4.04± 0.32* *
APC	VIR-2218- GL	6.81 ±0.6 3	7.20 ±0.1 5	6.16± 0.78	6.03± 0.76	6.22± 0.88	6.59± 0.46	7.07± 0.31	7.31± 0.27	7.43± 0.47	7.92± 0.29
B157S	C157S- DV27PG5	6.87 ±0.4 3	7.04 ±0.6 6	6.78± 0.48	6.60± 0.70	7.07± 0.40	6.95± 0.44	7.15± 0.52	7.09± 1.14	7.34± 0.58	7.86± 0.44
B207S	C207S- DV29PG5	6.98 ±0.2 3	7.07 ±0.3 9	6.51± 0.25	6.29± 0.33	5.95± 0.19	5.56± 0.36	5.70± 0.37	5.38± 0.40	5.78± 0.37	6.31± 0.44
B207S	C207S- DV32PG5	6.83 ±0.4 2	7.23 ±0.5 8	6.20± 0.35	5.59± 0.28	5.29± 0.43	4.79± 0.36	4.97± 0.26	4.83± 0.57	4.88± 0.36*	4.91± 0.38*

B418S	C418S-DV34Pd7BG5	6.68 ±0.7 8	6.71 ±0.7 5	6.39± 0.75	6.25± 0.88	6.84± 0.67	6.85± 0.91	7.21± 0.84	7.28± 1.00	7.58± 1.12	8.12± 0.89
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[0656] Note: Compared to the PBS group: * indicates $P < 0.05$; ** indicates $P < 0.01$.

[0657] Table 63: Changes in plasma HBeAg levels in mice across sequence groups

Unmodified sequence code	Sequence code	Log10 HBeAg (S/CO)									
		D-7	D0	D7	D14	D21	D28	D35	D42	D49	D56
/	PBS	3.76 ±0.1 7	3.73 ±0.1 0	3.61± 0.07	3.69 ±0.1 3	3.67 ±0.1 4	3.69 ±0.1 4	3.66 ±0.1 5	3.65 ±0.1 3	3.62 ±0.1 9	3.24 ±1.0 1
/	ETV	3.77 ±0.0 6	3.73 ±0.0 6	3.72± 0.05	3.72 ±0.0 3	3.66 ±0.0 5	3.71 ±0.0 6	3.66 ±0.0 5	3.68 ±0.0 5	3.69 ±0.0 8	3.70 ±0.0 4
APC	VIR-2218-GL	3.78 ±0.1 1	3.77 ±0.1 3	3.35± 0.15*	3.39 ±0.1 5	3.32 ±0.1 9	3.39 ±0.1 9	3.46 ±0.1 7	3.52 ±0.1 2	3.60 ±0.1 3	3.59 ±0.1 3
B1575	C1575-DV27PG5	3.75 ±0.1 9	3.68 ±0.1 3	3.48± 0.12	3.51 ±0.1 8	3.43 ±0.2 0	3.50 ±0.1 5	3.51 ±0.1 7	3.37 ±0.3 8	3.60 ±0.1 6	3.59 ±0.2 0
B207S	C207S-DV29PG5	3.78 ±0.0 6	3.70 ±0.0 7	3.50± 0.13	3.47 ±0.0 9	3.31 ±0.1 2	3.20 ±0.1 3	3.07 ±0.1 1	3.06 ±0.1 5	3.00 ±0.1 4	3.05 ±0.1 8
B207S	C207S-DV32PG5	3.77 ±0.0 4	3.72 ±0.0 4	3.45± 0.04* *	3.40 ±0.1 2	3.08 ±0.1 3	2.97 ±0.1 2	2.84 ±0.1 0*	2.94 ±0.3 5	2.73 ±0.1 1*	2.77 ±0.1 3
B418S	C418S-DV34Pd7BG5	3.74 ±0.3 0	3.66 ±0.2 6	3.38± 0.33	3.49 ±0.3 1	3.46 ±0.3 0	3.55 ±0.3 3	3.49 ±0.3 4	3.51 ±0.3 1	3.52 ±0.3 2	3.59 ±0.2 8

[0658] Note: Compared to the PBS group: * indicates $P < 0.05$; ** indicates $P < 0.01$.

[0659] Table 64: Number of mice with HBsAg clearance in plasma across sequence groups

Unmodified sequence code	Sequence code	Number of mice with HBsAg clearance							
		D7	D14	D21	D28	D35	D42	D49	D56
B207S	C207S-DV32PG5	0	0	0	1	1	2	1	3
B207S	C207S-DV29PG5	0	0	0	0	0	0	0	0
B418S	C418S-DV34Pd7BG5	0	0	0	0	0	0	0	0
B1575	C1575-DV27PG5	0	0	0	0	0	0	0	0
APC	VIR-2218-GL	0	0	0	0	0	0	0	0

/	ETV	0	0	0	0	0	0	0	0
/	PBS	0	0	0	0	0	0	0	0

[0660] Table 65: Number of mice with HBV DNA clearance in plasma across sequence groups

Unmodified sequence code	Sequence code	Number of mice with HBV DNA clearance							
		D7	D14	D21	D28	D35	D42	D49	D56
B207S	C207S-DV32PG5	0	0	0	0	0	0	0	0
B207S	C207S-DV29PG5	0	0	0	0	0	0	0	0
B418S	C418S-DV34Pd7BG5	0	0	0	0	0	0	0	0
B1575	C1575-DV27PG5	0	0	0	0	0	0	0	0
APC	VIR-2218-GL	0	0	0	0	0	0	0	0
/	ETV	1	1	1	3	3	2	0	0
/	PBS	0	0	0	0	0	0	0	0

[0661] Table 66: Number of mice producing HBsAb across sequence groups

Unmodified sequence code	Sequence code	Number of mice producing HBsAb							
		D7	D14	D21	D28	D35	D42	D49	D56
B207S	C207S-DV32PG5	1	1	2	3	4	4	4	4
B207S	C207S-DV29PG5	0	0	0	0	2	3	3	2
B418S	C418S-DV34Pd7BG5	0	0	0	0	0	0	0	0
B1575	C1575-DV27PG5	0	1	1	1	1	1	1	0
APC	VIR-2218-GL	0	0	0	0	0	0	0	0
/	ETV	0	0	0	0	0	0	0	0
/	PBS	0	0	0	0	0	0	0	0

[0662] Table 67: Changes in plasma HBsAb levels in mice across sequence groups

Unmodified sequence code	Sequence code	HBsAb (mIU/mL)							
		D7	D14	D21	D28	D35	D42	D49	D56
B207S	C207S-DV32PG5	45	79	135	136	124	109	130	122
B207S	C207S-DV29PG5	30	30	30	30	36	44	52	44
B418S	C418S-DV34Pd7BG5	30	30	30	30	30	30	30	30
B1575	C1575-DV27PG5	30	32	38	36	33	34	35	30
APC	VIR-2218-GL	30	30	30	30	30	30	30	30
	ETV	30	30	30	30	30	30	30	30
	PBS	30	30	30	30	30	30	30	30

[0663] Table 68: Changes in plasma ALT levels in mice across sequence groups

Sequence code	ALT (U/L)
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Unmodified sequence code		D 7	D1 4	D2 1	D2 8	D3 5	D4 2	D4 9	D5 6
B207S	C207S-DV32PG5	75	80	90	75	78	75	75	85
B207S	C207S-DV29PG5	75	78	80	75	100	93	75	75
B418S	C418S-DV34Pd7BG5	75	75	75	75	75	85	75	75
B1575	C1575-DV27PG5	75	75	75	75	75	75	75	75
APC	VIR-2218-GL	80	75	75	75	75	75	75	75
	ETV	75	75	75	75	75	75		
	PBS	75	75	75	75	75	75		

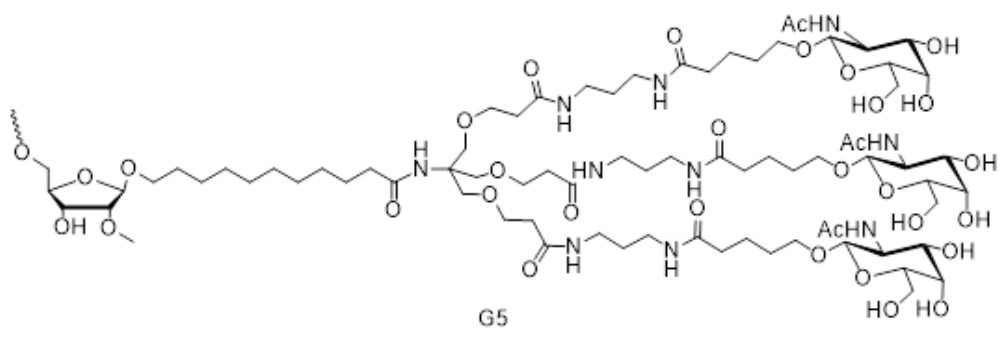
[0664] Example 9: Inhibition of HBV in mice using sequences modified with delivery ligands of the present disclosure

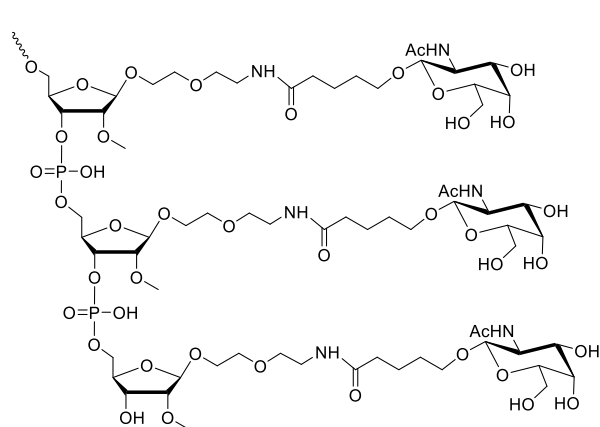
[0665] This example exemplarily selects certain sequences, including the unmodified sequences B418S and B207S, which are modified in various ways, such as template modification alone or template modification combined with anti-off-target design, and conjugated with different delivery ligands. The in vivo efficacy of these RNAi agents was analyzed using an AAV-HBV mouse model.

[0666] 1. Experimental materials

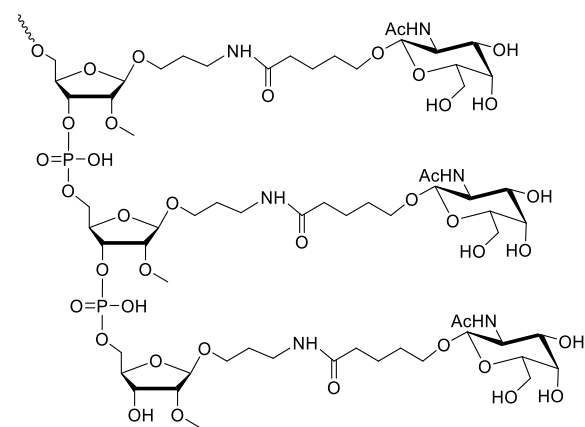
[0667] Test compounds:

[0668] The sequences listed in Table 69 include sequences with only template modifications and sequences with both template modifications and anti-off-target designs. The 3' end of the sense strand of these sequences is conjugated with delivery ligands G5, G101, or G103, with the structural formula as follows:





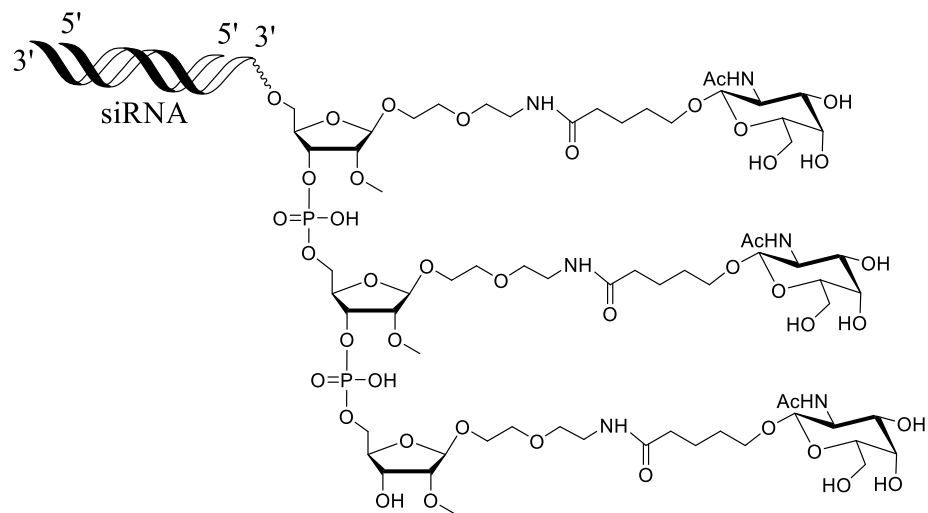
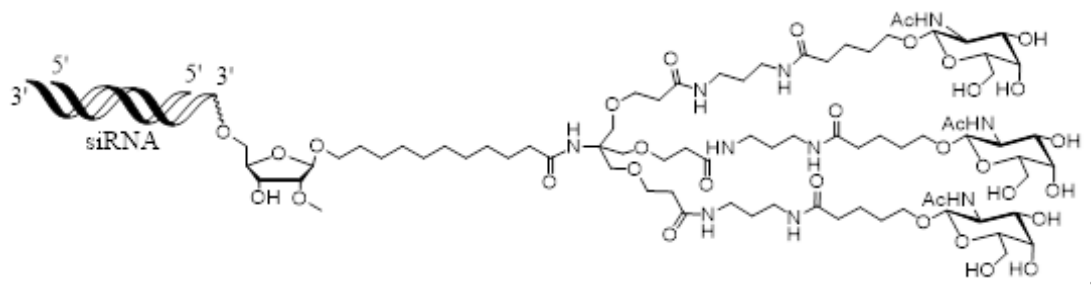
G101

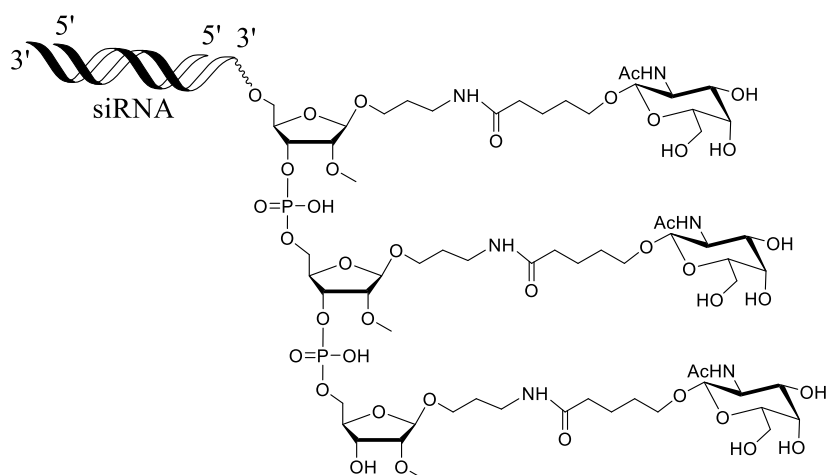


G103

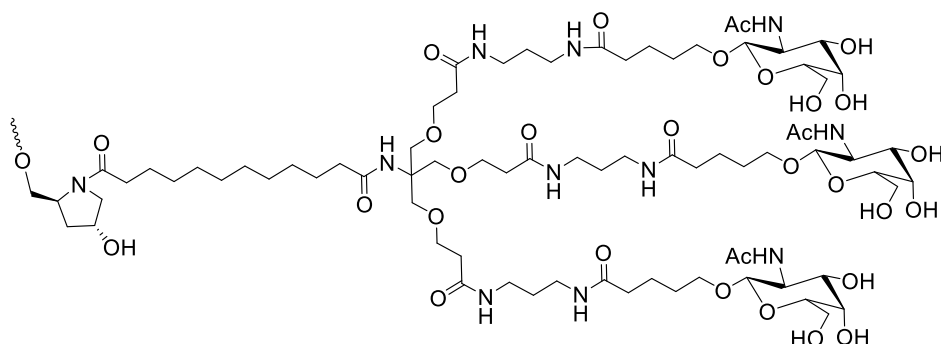
[0669] The conjugation method of the oligonucleotide with the ligand G5, G101 or G103 refers to the preparation method described in Example 3 of the patent application CN116854754A.

[0670] The oligonucleotide and ligand G5, G101 or G103 form the conjugate as shown below:





[0671] The specific sequences for each sequence can be found in Table 69. In the sequence codes, G5 indicates that the sequence is conjugated with the delivery ligand G5, G101 indicates conjugation with the delivery ligand G101, G103 indicates conjugation with the delivery ligand G103, and GL indicates conjugation with the delivery ligand L96. The structural formula of L96 is as follows:



[0672] Table 69: siRNA sequences used in animal experiments

Unmodified sequence code	Sequence code	Sense strand sequence 5'-3'	Sequence ID No	Unmodified sequence ID No	Antisense strand sequence 5'-3'	Sequence ID No	Unmodified sequence ID No
B207S	C207S - DV29 PG5	Gms-Gms-Gm-Gm-Um-Uf-Um-Uf-Um-Cf-Um-Um-Gm-Um-Um-Gf-Am-Cm-Am-Am-G5	326	1	UmsEVP-Ufs-Gm-Uf-Cf-Af-Am-Cm-Am-Am-Gm-Am-Am-Af-Am-Af-Cm-Cm-Cm-Cms-Gms-Cm	179	8
B207S	C207S - DV29 PG101	Gms-Gms-Gm-Gm-Um-Uf-Um-Uf-Um-Cf-Um-Um-Gm-Um-Um-Gf-Am-Cm-Am-Am-G101	327	1	UmsEVP-Ufs-Gm-Uf-Cf-Af-Am-Cm-Am-Am-Gm-Am-Am-Af-Am-Af-Cm-Cm-Cm-Cms-Gms-Cm	179	8

B207S	C207S - DV29 PG10 3	Gms-Gms-Gm-Gm-Um- Uf-Um-Uf-Um-Cf-Um- Um-Gm-Um-Um-Gf-Am- Cm-Am-Am-G103	328	1	UmsEVP-Ufs-Gm-Uf-Cf- Af-Am-Cm-Am-Am-Gm- Am-Am-Af-Am-Af-Cm- Cm-Cm-Cms-Gms-Cm	179	8
B207S	C207S - DV32 PG5	Gms-Gms-Gm-Gm-Um- Uf-Um-Uf-Um-Cf-Um- Um-Gm-Um-Um-Gf-Am- Cm-Am-Am-G5	329	1	UmsEVP-Ufs-Gm-Um-Cf- Af-Am-Cm-Am-Am-Gm- Am-Am-Af-Am-Af-Cm- Cm-Cm-Cms-Gms-Cm	181	8
B207S	C207S - DV32 PG10 1	Gms-Gms-Gm-Gm-Um- Uf-Um-Uf-Um-Cf-Um- Um-Gm-Um-Um-Gf-Am- Cm-Am-Am-G101	330	1	UmsEVP-Ufs-Gm-Um-Cf- Af-Am-Cm-Am-Am-Gm- Am-Am-Af-Am-Af-Cm- Cm-Cm-Cms-Gms-Cm	181	8
B207S	C207S - DV32 PG10 3	Gms-Gms-Gm-Gm-Um- Uf-Um-Uf-Um-Cf-Um- Um-Gm-Um-Um-Gf-Am- Cm-Am-Am-G103	331	1	UmsEVP-Ufs-Gm-Um-Cf- Af-Am-Cm-Am-Am-Gm- Am-Am-Af-Am-Af-Cm- Cm-Cm-Cms-Gms-Cm	181	8
B418S	C418S - DV34 Pd7B G5	Cms-Ums-Gm-Cm-Um- Af-Um-Gf-Cm-Cf-Um- Cm-Am-dT-Cm-Uf-Um- Cm-Um-Um-G5	350	6	AmsEVP-Afs-Gf-Am-Am- Gf-dA-Um-Gm-Am-Gm- Gm-Cm-Af-Um-Af-Gm- Cm-Am-Gms-Cms-Am	321	13
B418S	C418S - DV34 Pd7B G101	Cms-Ums-Gm-Cm-Um- Af-Um-Gf-Cm-Cf-Um- Cm-Am-dT-Cm-Uf-Um- Cm-Um-Um-G101	351	6	AmsEVP-Afs-Gf-Am-Am- Gf-dA-Um-Gm-Am-Gm- Gm-Cm-Af-Um-Af-Gm- Cm-Am-Gms-Cms-Am	321	13
B418S	C418S - DV34 Pd7B G103	Cms-Ums-Gm-Cm-Um- Af-Um-Gf-Cm-Cf-Um- Cm-Am-dT-Cm-Uf-Um- Cm-Um-Um-G103	352	6	AmsEVP-Afs-Gf-Am-Am- Gf-dA-Um-Gm-Am-Gm- Gm-Cm-Af-Um-Af-Gm- Cm-Am-Gms-Cms-Am	321	13
APC	VIR- 2218- GL	Gms-Ums-Gm-Um-Gf- Cm-Af-Cf-Uf-Um-Cm- Gm-Cm-Um-Um-Cm- Am-Cm-Am-GL	353	45	Ums-Gfs-Um-Gm-Am- A(gna)-Gm-Cf-Gf-Am-Am- Gm-Um-Gf-Cm-Af-Cm- Am-Cms-Ums-Um	294	79
ANC	CNC- G5	Gms-Gms-Um-Cm-Um- Cm-Uf-Um-Cf-Um-Af- Um-Cm-Gm-Am-Um- Cm-Um-Um-Am-Um-G5	354	46	AmsEVP-Ufs-Am-Af-Gf- Af-Um-Cm-Gm-Am-Um- Am-Gm-Af-Am-Gf-Am- Gm-Am-Cm-Cms-Cms-Am	295	80

[0673] Preparation of test compounds:

[0674] The procedure is the same as in Example 7.

[0675] Experimental animal information:

[0676] Species/Strain: AAV-HBV mice

[0677] Grade: SPF (Specific Pathogen-Free)

[0678] Gender: Male

[0679] Quantity: 82

[0680] Age: 5 weeks

[0681] Weight: 19–24 g

[0682] Source: Beijing Vital River Laboratory Animal Technology Co., Ltd.

[0683] Production License Number: SCXK (Jing) 2021-0006

[0684] Institutional Animal Care and Use Committee (IACUC):

[0685] After receipt, the experimental animals were feeded at Beijing Vitalstar Biotechnology Co., Ltd., under the license number: SCXK (Jing) 2022-0013. This project has been reviewed and approved by the Experimental Animal Ethics Committee of Beijing Vitalstar Biotechnology Co., Ltd., with the IACUC number VST-SY-24062701. The experimental procedures were strictly conducted in accordance with IACUC requirements to ensure animal welfare.

[0686] Feeding and management:

[0687] The animals were housed in a negative-pressure barrier environment using plastic (polycarbonate) cages (dimensions: 370 × 157 × 180 mm) in individually ventilated caging (IVC) systems. Due to the aggressive nature of male animals, they were housed individually. The feeding and management of the animals were the responsibility of Beijing Vitalstar Biotechnology Co., Ltd.

[0688] The use and testing of animal feed, bedding, and drinking water complied with the GB14925-2010 "Laboratory Animal—Requirements of Environment and Housing Facilities" standards. Environmental controls for animal feeding, including temperature, humidity, pressure differential, noise, illumination, air exchange rate, and ammonia concentration, were for now implemented in accordance with the GB50447-2008 "Technical Specification for Construction of Laboratory Animal Facilities." The animal room temperature was maintained at 20–26°C (daily temperature variation ≤ 4°C), and relative humidity was controlled at 40–

70%. Artificial lighting was used to provide a 12/12-hour light/dark cycle. Compressed wood shavings for bedding were purchased from Beijing Ke'ao Xieli Feed Co., Ltd.(Batch No.: 23109613). Rodent growth and breeding feed were purchased from Beijing Ke'ao Xieli Feed Co., Ltd. (Batch No.: 23103313). All records related to animal care, management, and environmental controls during the experimental period were maintained and stored at Beijing Vitalstar Biotechnology Co., Ltd.

[0689] 2. Experimental methods

[0690] 2.1 AAV-HBV mouse model preparation

[0691] Definition of experimental timeline: The day of administration of the vehicle or test substance to the animals is defined as Day 0.

[0692] One hundred SPF-grade C57BL/6 male mice were acclimatized in a barrier facility for 7 days, with daily observations to confirm their health and absence of abnormalities before model preparation. A persistent HBV-infected mouse model was established by tail vein injection of rAAV8-1.3HBV. The AAV virus injection dose was 1.00×10^{10} vg/mouse. The AAV virus was diluted with sterile PBS to a concentration of 5.00×10^{10} vg/mL, and each mouse was injected with 200 μ L. Serum levels of HBV DNA, HBeAg, and HBsAg were measured starting 4 weeks after virus injection.

[0693] 2.2 Animal grouping and administration

[0694] Definition of experimental timeline: The day of administration of the vehicle or test substance to the animals is defined as Day 0 (D0).

[0695] On Day -2, blood was collected from all mice via the submandibular vein to obtain plasma. The collected blood samples were anticoagulated with EDTA, centrifuged at 5000 rpm for 10 minutes, and the supernatant was used for HBV model validation. On Day 0, based on the results from Day -2, 72 animals were selected and divided into 12 groups, with 6 mice in each group. Details on grouping and administration are as shown in Table 70.

[0696] Table 70: Group settings and administration details

Group	Administration regimen	Administration route	Number of animals
siRNA (11 groups)	3 mg/kg, administered on D0	s.c.	6 mice/group $\times$ 11 group

Vehicle control (1 group)	PBS, administered once on D0	s.c.	6 mice/group × 1 group
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[0697] Note: s.c. represents subcutaneous injection.

[0698] 2.3 Observation and indicator monitoring

[0699] (1) General observation

[0700] During both the model preparation and experimental periods, animals were observed daily, and records were maintained. Observations included: mortality, moribund state, feed and water intake, injuries, feces, appearance and coat condition, mental state, and activity levels.

[0701] (2) Body weight

[0702] Acclimatization period: Body weights were recorded upon animal receipt and on the final day of the acclimatization period.

[0703] Experimental period: Body weights were recorded weekly during the experimental period. If administration or blood collection was scheduled on the same day, weights were measured prior to the procedure or before euthanasia.

[0704] (3) Measurement of serum HBsAg, HBeAg, HBV DNA, and ALT levels

[0705] Blood was collected from all animals via the submandibular vein on Days -2, 6, 13, 20, 27, and 34 to obtain plasma. The collected blood samples were anticoagulated with EDTA, centrifuged at 5000 rpm for 10 minutes, and the serum was separated. After blood collection, the serum was separated, diluted with PBS, and sent for testing. For each sample, 10 μ L of serum was diluted to 500 μ L with PBS (50-fold dilution) and sent to Beijing Di'an Medical Laboratory Co., Ltd. for the detection of serum HBV DNA, HBeAg, and HBsAg. For each sample, 30 μ L of serum was diluted to 120 μ L with PBS (4-fold dilution) and sent to Beijing Di'an Medical Laboratory Co., Ltd. for the detection of serum ALT. The remaining plasma was stored at -80°C for future use.

[0706] 3. Experimental results

[0707] The changes in HBsAg, HBeAg, HBV DNA, ALT levels, and body weight over time for each group of mice are shown in Tables 71-75 and FIGs 4A-4E. The experimental results indicate that the efficacy of all tested sequences was superior to that of the positive control sequence VIR-2218-GL. Among them, the sequences C207S-DV29PG101, C207S-DV29PG103, C207S-DV32PG101, and C207S-DV32PG103 demonstrated superior efficacy

in reducing HBsAg, HBV DNA, and HBeAg levels, significantly outperforming the positive control sequence VIR-2218-GL. By Day 28 post-administration, these sequences still reduced HBsAg by more than 3 log₁₀ and HBV DNA by more than 3 log₁₀, with no rebound observed, demonstrating excellent anti-HBV efficacy.

[0708] Table 71: Changes in plasma HBsAg levels in mice across sequence groups

Unmodified sequence code	Sequence code	Log ₁₀ HBsAg (IU/mL)						
		D-2	D7	D14	D21	D28	D35	D42
/	PBS	4.47±0.12	4.38±0.15	4.36±0.21	4.26±0.27	4.3±0.21	4.37±0.17	4.22±0.16
B207S	C207S-DV29PG5	4.46±0.13	3.11±0.67	2.54±0.79	2.16±0.8	1.94±0.9	1.78±0.95	1.56±0.98
B207S	C207S-DV29PG101	4.43±0.16	2.65±0.32	1.8±0.59	1.47±0.7	1.44±0.69	1.19±0.77	1±0.83
B207S	C207S-DV29PG103	4.46±0.15	2.54±0.37	1.66±0.47	1.36±0.58	1.22±0.71	1.04±0.71	1.02±0.73
B207S	C207S-DV32PG5	4.45±0.15	2.86±0.49	2.34±0.7	2.08±0.66	1.88±0.74	1.75±0.83	1.56±1
B207S	C207S-DV32PG101	4.46±0.12	2.5±0.2	1.66±0.22	1.47±0.31	1.27±0.39	1.12±0.51	1.19±0.43
B207S	C207S-DV32PG103	4.42±0.22	2.25±0.64	1.33±0.64	1.3±0.59	1.21±0.53	1.18±0.55	1.18±0.62
B418S	C418S-DV34Pd7BG5	4.43±0.15	2.92±0.19	2.99±0.19	3.23±0.31	3.58±0.31	3.83±0.22	3.81±0.42
B418S	C418S-DV34Pd7BG101	4.47±0.11	2.67±0.28	2.35±0.54	2.63±0.59	2.95±0.57	3.21±0.52	3.24±0.6
B418S	C418S-DV34Pd7BG103	4.46±0.11	2.68±0.55	2.51±0.56	2.69±0.51	2.94±0.6	3.26±0.46	3.52±0.35
APC	VIR-2218-GL	4.46±0.14	3.66±0.24	3.71±0.23	3.84±0.37	3.86±0.39	3.87±0.41	3.82±0.45
ANC	CNC-G5	4.44±0.21	4.27±0.13	4.21±0.3	4.33±0.32	4.25±0.3	4.26±0.4	4.22±0.33

[0709] Table 72: Changes in plasma HBV DNA levels in mice across sequence groups

Unmodified sequence code	Sequence code	Log ₁₀ HBV DNA (IU/mL)						
		D-2	D7	D14	D21	D28	D35	D42
/	PBS	8.47±0.27	8.58±0.21	8.68±0.28	8.71±0.38	8.57±0.53	8.73±0.46	8.7±0.64

B207S	C207S-DV29PG5	8.47± 0.23	7.46± 0.67	6.74± 0.97	6.32± 0.77	5.97± 1.43	6.01± 1.09	5.69± 1.16
B207S	C207S-DV29PG101	8.5±0. 17	7.21± 0.32	5.78± 0.51	5.53± 0.85	5.3±0. 94	5.33± 0.69	4.91± 1.22
B207S	C207S-DV29PG103	8.51± 0.25	6.8±0. 32	5.41± 0.51	5.48± 0.8	4.97± 1.14	4.98± 1.17	5.09± 1.09
B207S	C207S-DV32PG5	8.49± 0.21	7.5±0. 5	6.7±0. 74	6.24± 0.88	6.39± 0.78	6.29± 0.8	6.15± 0.78
B207S	C207S-DV32PG101	8.47± 0.27	6.88± 0.47	5.1±0. 5	5.3±1. 07	5.06± 1	5.24± 0.7	5.29± 0.61
B207S	C207S-DV32PG103	8.49± 0.28	6.84± 0.64	5.64± 0.73	5.31± 0.91	5.41± 0.67	5.25± 1.1	5.51± 0.86
B418S	C418S-DV34Pd7BG5	8.5±0. 15	7.57± 0.18	7.68± 0.26	8.02± 0.34	8.48± 0.37	8.77± 0.22	8.78± 0.47
B418S	C418S-DV34Pd7BG10 1	8.53± 0.12	7.17± 0.27	6.89± 0.69	7.12± 0.73	7.66± 0.67	7.94± 0.5	8.07± 0.68
B418S	C418S-DV10334Pd7B G	8.53± 0.2	7.13± 0.44	7.01± 0.58	7.18± 0.66	7.56± 0.65	7.94± 0.47	8.29± 0.38
APC	VIR-2218-GL	8.42± 0.3	8.24± 0.27	8.43± 0.31	8.62± 0.41	8.57± 0.47	8.49± 0.66	8.57± 0.57
ANC	CNC-G5	8.43± 0.29	8.58± 0.29	8.55± 0.29	8.76± 0.21	8.83± 0.31	8.72± 0.41	8.79± 0.33

[0710] Table 73: Changes in plasma HBeAg levels in mice across sequence groups

Unmodified sequence code	Sequence code	Log10 HBeAg (IU/mL)						
		D-2	D7	D14	D21	D28	D35	D42
/	PBS	3.43± 0.06	3.44± 0.02	3.46± 0.03	3.45± 0.03	3.39± 0.06	3.43± 0.04	3.49± 0.03
B207S	C207S-DV29PG5	3.42± 0.03	3.18± 0.1	3.04± 0.17	2.81± 0.21	2.67± 0.24	2.59± 0.27	2.65± 0.28
B207S	C207S-DV29PG101	3.43± 0.02	3.18± 0.04	2.87± 0.14	2.58± 0.11	2.43± 0.1	2.39± 0.07	2.39± 0.1
B207S	C207S-DV29PG103	3.44± 0.04	3.22± 0.06	2.82± 0.08	2.61± 0.08	2.47± 0.1	2.46± 0.06	2.45± 0.06
B207S	C207S-DV32PG5	3.44± 0.03	3.24± 0.04	3.03± 0.09	2.77± 0.14	2.63± 0.12	2.6±0. 14	2.65± 0.15
B207S	C207S-DV32PG101	3.44± 0.03	3.17± 0.06	2.81± 0.1	2.58± 0.07	2.42± 0.06	2.39± 0.06	2.4±0. 06
B207S	C207S-DV32PG103	3.46± 0.03	3.19± 0.07	2.91± 0.1	2.69± 0.13	2.57± 0.12	2.52± 0.13	2.56± 0.12

B418S	C418S-DV34Pd7BG5	3.43±0.03	3.18±0.09	3.25±0.06	3.3±0.04	3.24±0.06	3.3±0.06	3.38±0.04
B418S	C418S-DV34Pd7BG101	3.41±0.05	3.19±0.07	3.25±0.09	3.22±0.07	3.18±0.07	3.2±0.08	3.3±0.06
B418S	C418S-DV10334Pd7BG	3.43±0.02	3.17±0.03	3.21±0.04	3.26±0.06	3.16±0.08	3.19±0.1	3.28±0.08
APC	VIR-2218-GL	3.4±0.07	3.09±0.07	3.2±0.09	3.29±0.05	3.25±0.08	3.27±0.1	3.35±0.17
ANC	CNC-G5	3.42±0.03	3.42±0.04	3.46±0.05	3.47±0.04	3.42±0.05	3.41±0.05	3.47±0.04

[0711] Table 74: Changes in plasma ALT levels in mice across sequence groups

Unmodified sequence code	Sequence code	ALT (U/L)					
		D7	D14	D21	D28	D35	D42
B207S	C207S-DV29PG5	30	53	111	68	71	41
	C207S-DV29PG101	50	45	129	83	50	61
	C207S-DV29PG103	44	63	129	57	49	35
	C207S-DV32PG5	51	49	122	71	43	40
	C207S-DV32PG101	41	61	103	61	48	43
	C207S-DV32PG103	29	45	146	56	49	33
B418S	C418S-DV34Pd7BG5	34	49	41	48	49	36
	C418S-DV34Pd7BG101	48	30	48	75	44	76
	C418S-DV34Pd7BG103	42	30	62	69	41	32
APC	VIR-2218-GL	47	35	37	79	50	49
CNC	CNC-G5	48	51	60	60	61	58
	PBS	51	53	49	75	65	59

[0712] Table 75: Changes in body weight of mice across sequence groups

Unmodified sequence code	Sequence code	Body weight (g)					
		D7	D14	D21	D28	D35	D42
B207S	C207S-DV29PG5	25.8	25.6	25.6	26	27.0	27.5
	C207S-DV29PG101	24.8	24.4	24.5	24.4	25.6	26.0
	C207S-DV29PG103	25.7	25.4	25.2	25.5	26.6	27.0
	C207S-DV32PG5	25.6	25.7	25.7	25.5	26.4	26.4
	C207S-DV32PG101	24.6	24.4	24.1	24.4	25.3	26.0
	C207S-DV32PG103	25	25	24.6	24.7	25.8	26.1
B418S	C418S-DV34Pd7BG5	25.6	25.7	25.8	26.2	26.6	27.1
	C418S-DV34Pd7BG101	25.4	25.6	25.4	25.9	26.8	27.4
	C418S-DV34Pd7BG103	26.1	26.2	26.5	27	27.8	28.5

APC	VIR-2218-GL	25.5	25.9	26	26.3	27.0	27.3
CNC	CNC-G5	25.4	25.5	25.5	26.4	26.9	27.1
	PBS	25.3	25.1	25.6	25.9	26.7	26.6

CLAIMS

1. A double-stranded RNAi agent, wherein the double-stranded RNAi agent comprises an antisense strand and a sense strand complementary to the antisense strand forming a double-stranded region, wherein a nucleotide sequence of the antisense strand is as shown in SEQ ID NO: 8, 11, or 13, or a nucleotide sequence of the antisense strand is a modified sequence of a sequence shown in SEQ ID NO: 8, 11, or 13.

2. The double-stranded RNAi agent according to claim 1, wherein the double-stranded RNAi agent comprises an oligonucleotide duplex formed by pairing of the sense strand and the antisense strand;

wherein the sense strand has a sequence as shown in SEQ ID NO: 1, 4, or 6, or a fragment thereof, or a modified sequence of the sequence or fragment thereof.

3. The double-stranded RNAi agent according to claim 2, wherein the sense strand and the antisense strand comprise at least one modified nucleotide.

4. The double-stranded RNAi agent according to claim 1, wherein the double-stranded RNAi agent has the function of inhibiting HBV gene expression.

5. The double-stranded RNAi agent according to claim 3, wherein at least one of the modified nucleotides is selected from one or more of the group consisting of: deoxy-nucleotide, 3'-terminal deoxy-thymidine nucleotide, 2'-O-methyl-modified nucleotide, 2'-fluoro-modified nucleotide, 2'-deoxy-modified nucleotide, locked nucleotide, unlocked nucleotide, conformationally restricted nucleotide, constrained ethyl nucleotide, abasic nucleotide, 2'-amino-modified nucleotide, 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxy-modified nucleotide, 2'-O-methoxyethyl-modified nucleotide, 2'-O-alkyl-modified nucleotide, morpholino nucleotide, phosphoramidate, nucleotide containing unnatural bases, tetrahydropyran-modified nucleotide, 1,5-anhydrohexitol-modified nucleotide, cyclohexenyl-modified nucleotide, nucleotide containing phosphorothioate groups, nucleotide containing methylphosphonate groups, nucleotide containing 5'-phosphate, and nucleotide containing 5'-

phosphate mimetics.

6. The double-stranded RNAi agent according to claim 1, wherein at least one strand of the double-stranded RNAi agent comprises a 3' overhang of at least 2 nucleotides.

7. The double-stranded RNAi agent according to claim 1, wherein the duplex region of the double-stranded RNAi agent is 20 nucleotide pairs in length.

8. The double-stranded RNAi agent according to claim 1, wherein the sense strand of the double-stranded RNAi agent has 20 nucleotides, and the antisense strand has 22 nucleotides.

9. The double-stranded RNAi agent according to claim 1, wherein all modifications on the nucleotides of the sense and antisense strands are chemical modifications at the 2' position of a nucleotide ribose.

10. The double-stranded RNAi agent according to claim 9, wherein the chemical modifications at the 2' position of the nucleotide ribose are selected from one or more of the group consisting of: 2'-methoxy, 2'-O-methoxyethyl, 2'-fluoro, 2'-benzyloxy, 2'-methylcarbonylamino, and 2'-pyridylmethoxy.

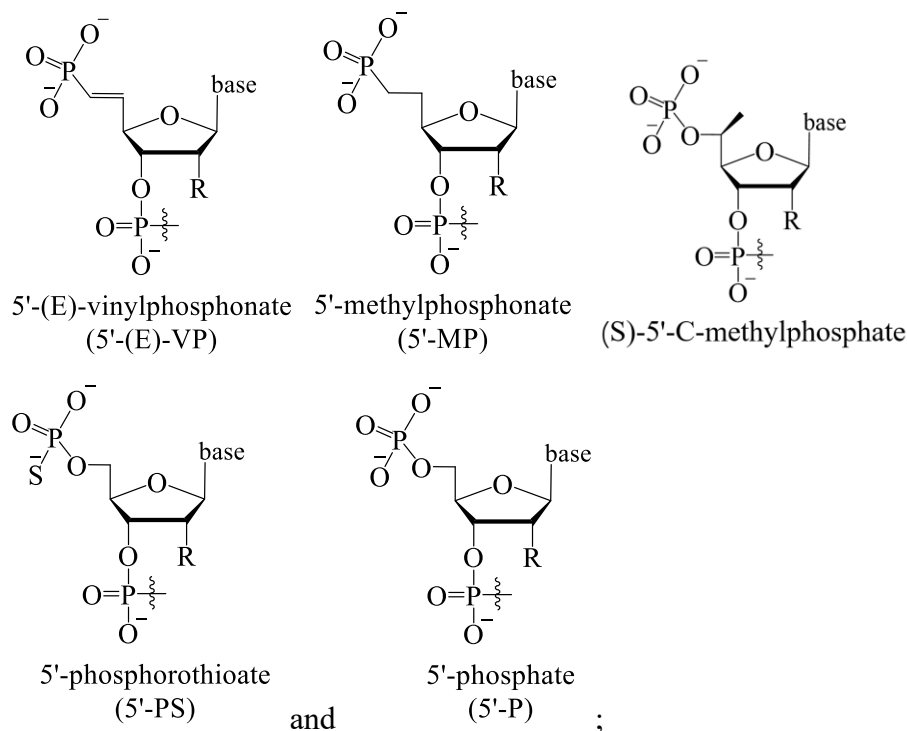
11. The double-stranded RNAi agent according to claim 10, wherein the chemical modification at the 2' position of the nucleotide ribose is 2'-methoxy or 2'-fluoro.

12. The double-stranded RNAi agent according to claim 1, wherein the nucleotides are linked by 3',5'-phosphodiester bonds.

13. The double-stranded RNAi agent according to claim 12, wherein the 3',5'-phosphodiester bond comprises a phosphorothioate modification.

14. The double-stranded RNAi agent according to claim 1, wherein a 5' carbon atom of the 5' terminal nucleotide glycoside of the antisense strand is phosphorylated.

15. The double-stranded RNAi agent according to claim 14, wherein the phosphorylated 5' phosphorylated group includes one or more selected from 5'-vinylphosphonate group, 5'-methylphosphonate group, 5'-C-methylphosphate group, 5'-phosphorothioate group, and 5'-phosphate group, with the structure being



R is hydrogen, hydroxyl, amino, C₁₋₄ alkyl, aryl, C₁₋₄ alkoxy, C₁₋₄ alkylcarbonylamino, or halogen;

the base is selected from any one of adenine, guanine, cytosine, thymine, and uracil.

16. The double-stranded RNAi agent according to claim 1, wherein terminal nucleotides of the sequence are linked by a 3',5'-phosphodiester bond containing a phosphorothioate modification, forming a chirally pure 3',5'-phosphorothioate bond.

17. The double-stranded RNAi agent according to claim 16, wherein a 5' end of the sense strand and the antisense strand comprises 1 to 3 phosphorothioate linkages, and a 3' end of the antisense strand comprises 1 to 3 phosphorothioate linkages.

18. The double-stranded RNAi agent according to claim 1, wherein the antisense strand

adopts one of the modification patterns outlined in the table below:

N o .	Antisense strand (AS) 5'-3'																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
A	2'-OMe+PS+EV P	2'-OMe+PS	2'-OMe	2'-F	2'-F	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
	2'-OMe+PS+EV P	2'-F+PS	2'-F	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
	2'-OMe+PS+EV P	2'-F+PS	2'-F	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
	2'-OMe+PS+EV P	2'-F+PS	2'-F	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
B	2'-OMe+PS+EV P	2'-F+PS	2'-F	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
	2'-OMe+PS+EV P	2'-F+PS	2'-F	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
	2'-OMe+PS+EV P	2'-F+PS	2'-F	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
	2'-OMe+PS+EV P	2'-F+PS	2'-F	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
C	2'-OMe+PS+EV P	2'-F+PS	2'-F	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
	2'-OMe+PS+EV P	2'-F+PS	2'-F	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
	2'-OMe+PS+EV P	2'-F+PS	2'-F	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
	2'-OMe+PS+EV P	2'-F+PS	2'-F	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
D	2'-OMe	2'-F	2'-F	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
	2'-OMe	2'-F	2'-F	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
	2'-OMe	2'-F	2'-F	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe
	2'-OMe	2'-F	2'-F	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-OMe	2'-F	2'-OMe	2'-F	2'-OMe	2'-OMe	2'-OMe	2'-OMe+PS	2'-OMe+PS	2'-OMe

	e + P S + E V P		e	e		e	e	e	e	e	e	e		e		e	e	e	PS	e + P S	e
E	2' - O M e + P S + E V P	2' - F + P S	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e + PS	2' - O M e + P S	2' - O M e
F	2' - O M e + P S + E V P	2' - F + P S	2' - F	2' - O M e	2' - F	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e + PS	2' - O M e + P S	2' - O M e

; or

N o .	Antisense strand (AS) 5'-3'																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
A	2' - O M e + P S	2' - F + P S	2' - O M e	2' - F	2' - F	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F	2' - O M e	2' - F	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e + PS	2' - O M e + P S	2' - O M e

	+ E V P																					
B	2'- O M e + P S + E V P	2' - F + P S	2' - F	2' - F	2'- O M e	2' - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2' - F	2'- O M e	2' - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e + P S	2'- O M e + P S	2'- O M e
C	2'- O M e + P S + E V P	2' - F + P S	2' - O M e	2' - O M e	2'- F	2' - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2' - F	2'- O M e	2' - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e + P S	2'- O M e + P S	2'- O M e
D	2'- O M e + P S + E V P	2' - F + P S	2' - F	2' - O M e	2'- O M e	2' - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2' - F	2'- O M e	2' - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e + P S	2'- O M e + P S	2'- O M e
E	2'- O M	2' - F	2' - O	2' - O	2'- O M	2' - F	2'- O M	2'- O M	2'- O M	2'- O M	2'- O M	2'- O M	2'- O M	2' - F	2'- O M	2' - F	2'- O M	2'- O M	2'- O M	2'- O M e +	2'- O M	2'- O M

	e + P S + E V P	+ P S	M e	M e	e		e	e	e	e	e	e	e		e		e	e	e	e	PS	e + P S	e
F	2'- O M e + P S + E V P	2'- - F + P S	2'- - F	2'- - O M e	2'- - F	2'- - F	2'- - O M e	2'- - O M e	2'- - O M e	2'- - O M e	2'- - O M e	2'- - O M e	2'- - O M e	2'- - F	2'- - O M e	2'- - F	2'- - O M e	2'- - O M e	2'- - O M e	2'- - O M e	2'- O M e + PS	2'- - O M e + P S	2'- - O M e

and/or, the sense strand adopts one of the modification patterns outlined in the table below:

N o .	Sense strand (SS) 5'-3'																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
a	2'- O M e + P S	2'- O M e + P S	2'- O M e	2'- O M e	2'- O M e	2'- - F	2'- O M e	2'- - F	2'- - F	2'- - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e
b	2'- O M e + P S	2'- O M e + P S	2'- O M e	2'- O M e	2'- O M e	2'- - F	2'- O M e	2'- - F	2'- O M e	2'- - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- O M e	2'- - F	2'- O M e	2'- O M e	2'- O M e	2'- O M e

or

N	Sense strand (SS) 5'-3'
---	-------------------------

o .	1	2	3	4	5	6	7	8	9	1 0	1 1	1 2	1 3	1 4	1 5	1 6	1 7	1 8	1 9	2 0	2 1
a	2' - O M e + P S	2' - O M e + P S	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F M e	2' - O M e	2' - F M e	2' - F M e	2' - F M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e
b	2' - O M e + P S	2' - O M e + P S	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F M e	2' - O M e	2' - F M e	2' - O M e	2' - F M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e	2' - F M e	2' - O M e	2' - O M e	2' - O M e	2' - O M e

wherein 2'-OMe is 2'-methoxy; 2'-F is 2'-fluoro; PS is phosphorothioate backbone; EVP is 5'-(E)-vinylphosphonate.

19. The double-stranded RNAi agent according to claim 18, wherein the modification patterns of the double-stranded RNAi agent are as follows:

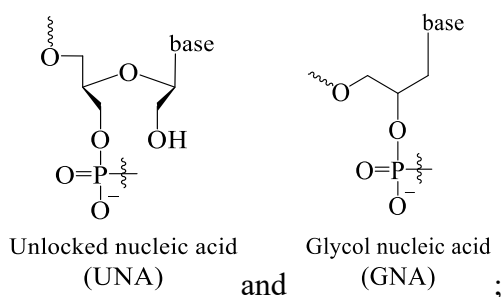
the antisense strand is modified by pattern A and the sense strand modified by pattern a;
the antisense strand is modified by pattern A and the sense strand modified by pattern b;
the antisense strand is modified by pattern B and the sense strand modified by pattern a;
the antisense strand is modified by pattern B and the sense strand modified by pattern b;
the antisense strand is modified by pattern C and the sense strand modified by pattern b;
the antisense strand is modified by pattern D and the sense strand modified by pattern b;
the antisense strand is modified by pattern E and the sense strand modified by pattern a;

or

the antisense strand is modified by pattern F and the sense strand modified by pattern b.

20. The double-stranded RNAi agent according to claim 1, wherein the antisense strand is modified with modifying groups from the second to the eighth positions starting from the 5' end, where the modifying groups are selected from one or more of UNA, GNA, and DNA, and

the structures of UNA and GNA are



the base is selected from any one of adenine, guanine, cytosine, thymine, and uracil.

21. The double-stranded RNAi agent according to any one of claims 1 to 20, wherein the double-stranded RNAi agent comprises an oligonucleotide duplex selected from any one of the following sense and antisense strand pairs:

(1) the sense strand has the sequence as shown in SEQ ID NO: 81, and the antisense strand has the sequence as shown in SEQ ID NO: 179, 180, 181, 182, or 184;

(2) the sense strand has the sequence as shown in SEQ ID NO: 82, and the antisense strand has the sequence as shown in SEQ ID NO: 179, 180, or 183;

(3) the sense strand has the sequence as shown in SEQ ID NO: 83, and the antisense strand has the sequence as shown in SEQ ID NO: 185 or 186;

(4) the sense strand has the sequence as shown in SEQ ID NO: 84, and the antisense strand has the sequence as shown in SEQ ID NO: 186;

(5) the sense strand has the sequence as shown in SEQ ID NO: 85, and the antisense strand has the sequence as shown in SEQ ID NO: 186;

(6) the sense strand has the sequence as shown in SEQ ID NO: 96, and the antisense strand has the sequence as shown in SEQ ID NO: 201, 202, 203, or 204;

(7) the sense strand has the sequence as shown in SEQ ID NO: 97, and the antisense strand has the sequence as shown in SEQ ID NO: 201 or 202;

(8) the sense strand has the sequence as shown in SEQ ID NO: 98, and the antisense strand has the sequence as shown in SEQ ID NO: 205 or 206;

(9) the sense strand has the sequence as shown in SEQ ID NO: 99, and the antisense strand has the sequence as shown in SEQ ID NO: 206;

(10) the sense strand has the sequence as shown in SEQ ID NO: 100, and the antisense strand has the sequence as shown in SEQ ID NO: 206;

(11) the sense strand has the sequence as shown in SEQ ID NO: 106, and the antisense strand has the sequence as shown in SEQ ID NO: 213, 214, 215, or 216;

(12) the sense strand has the sequence as shown in SEQ ID NO: 107, and the antisense strand has the sequence as shown in SEQ ID NO: 214 or 213;

(13) the sense strand has the sequence as shown in SEQ ID NO: 108, and the antisense strand has the sequence as shown in SEQ ID NO: 217 or 218;

(14) the sense strand has the sequence as shown in SEQ ID NO: 109, and the antisense strand has the sequence as shown in SEQ ID NO: 218;

(15) the sense strand has the sequence as shown in SEQ ID NO: 110, and the antisense strand has the sequence as shown in SEQ ID NO: 218;

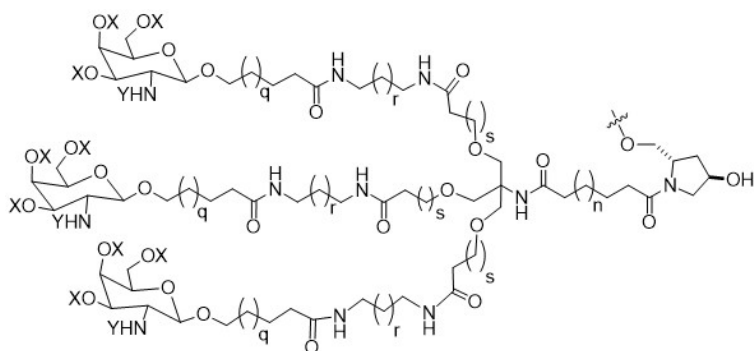
(16) the sense strand has the sequence as shown in SEQ ID NO: 306, and the antisense strand has the sequence as shown in SEQ ID NO: 321.

22. A conjugate, wherein the conjugate comprises the double-stranded RNAi agent according to any one of claims 1 to 21 and a ligand conjugated to the double-stranded RNAi agent.

23. The conjugate according to claim 22, wherein the ligand is conjugated to a 3'-end or a 5'-end of an oligonucleotide sense strand.

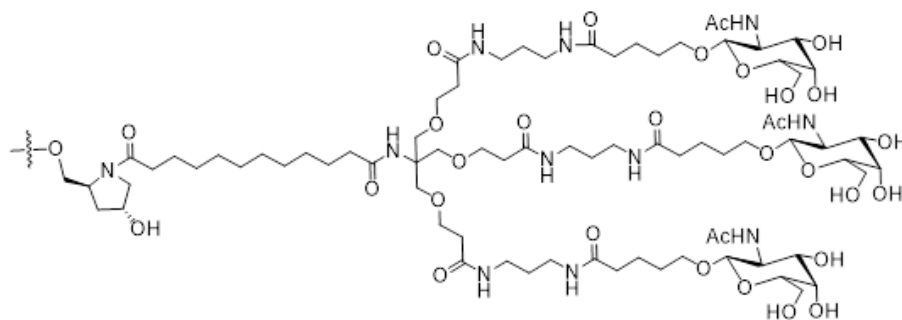
24. The conjugate according to claim 22, wherein the ligand is one or more GalNAc derivatives attached via a bivalent or trivalent branched linker, or a GalNAc derivative attached via a monovalent linker.

25. The conjugate according to claim 22, wherein the ligand is:

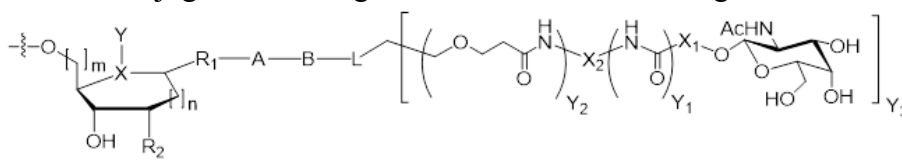


wherein X is hydrogen, a hydroxyl protecting group, or H, and the hydroxyl protecting group includes acetyl, benzoyl, or isobutyryl; Y is an amine protecting group or H, and the amine protecting group is formyl, acetyl, propionyl, n-butyryl, or isobutyryl; n is an integer between 0 and 20; q, r, and s are independently integers between 1 and 7.

26. The conjugate according to claim 25, wherein the ligand is:



27. The conjugate according to claim 22, wherein the ligand is:



wherein X is oxygen, nitrogen, or sulfur;

Y is an alkyl or aromatic group;

R₁ is oxygen or sulfur;

R₂ is hydrogen, an amino group, a C₁₋₄ alkyl group, an aromatic group, a C₁₋₄ alkoxy group, or a halogen;

A is -(CH₂)_a-, -(CH₂CH₂O)_b-, -((CH₂)_cNHCO)_d-, or -((CH₂)_cCONH)_d-, where a is an integer from 1 to 15, b is an integer from 1 to 7, c is an integer from 1 to 7, and d is an integer from 1 to 5;

B is -(CH₂)_e-, where e is an integer from 0 to 7;

L is -CONH- or -NHCO-;

X₁ is -(CH₂)_f- or -(CH₂CH₂O)_fCH₂-, where f is an integer from 1 to 5;

X₂ is -(CH₂)_g-, where g is an integer from 1 to 6;

Y₁ is 0 or 1;

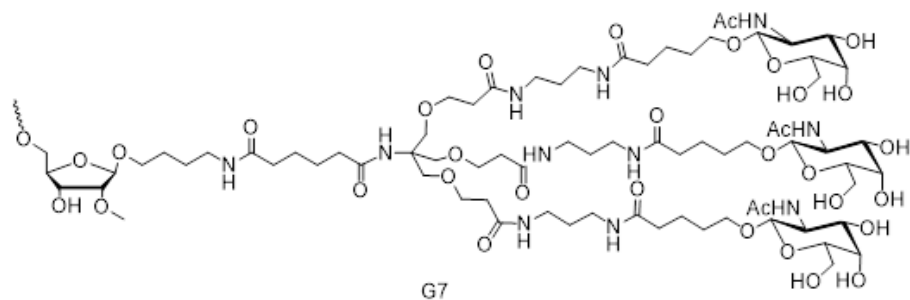
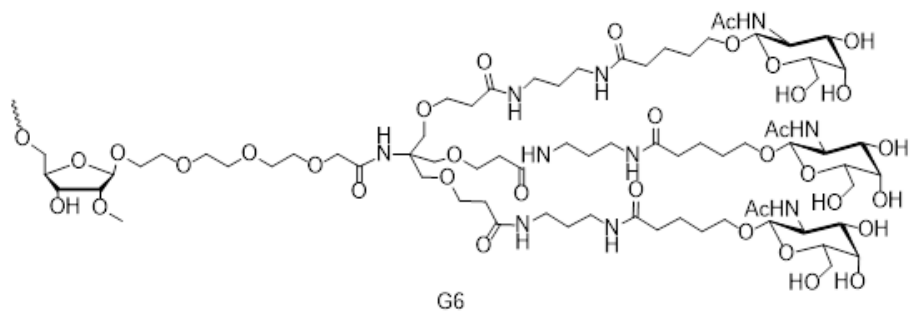
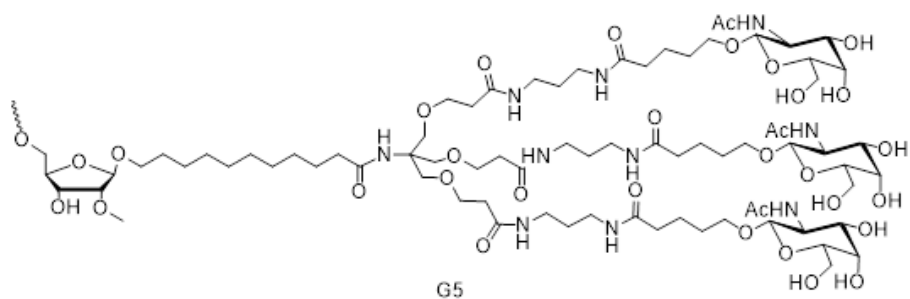
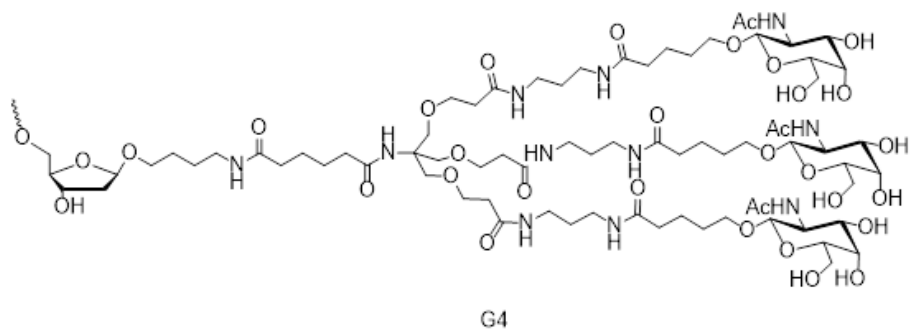
Y₂ is 0, 1, or 2;

Y₃ is 1, 2, or 3;

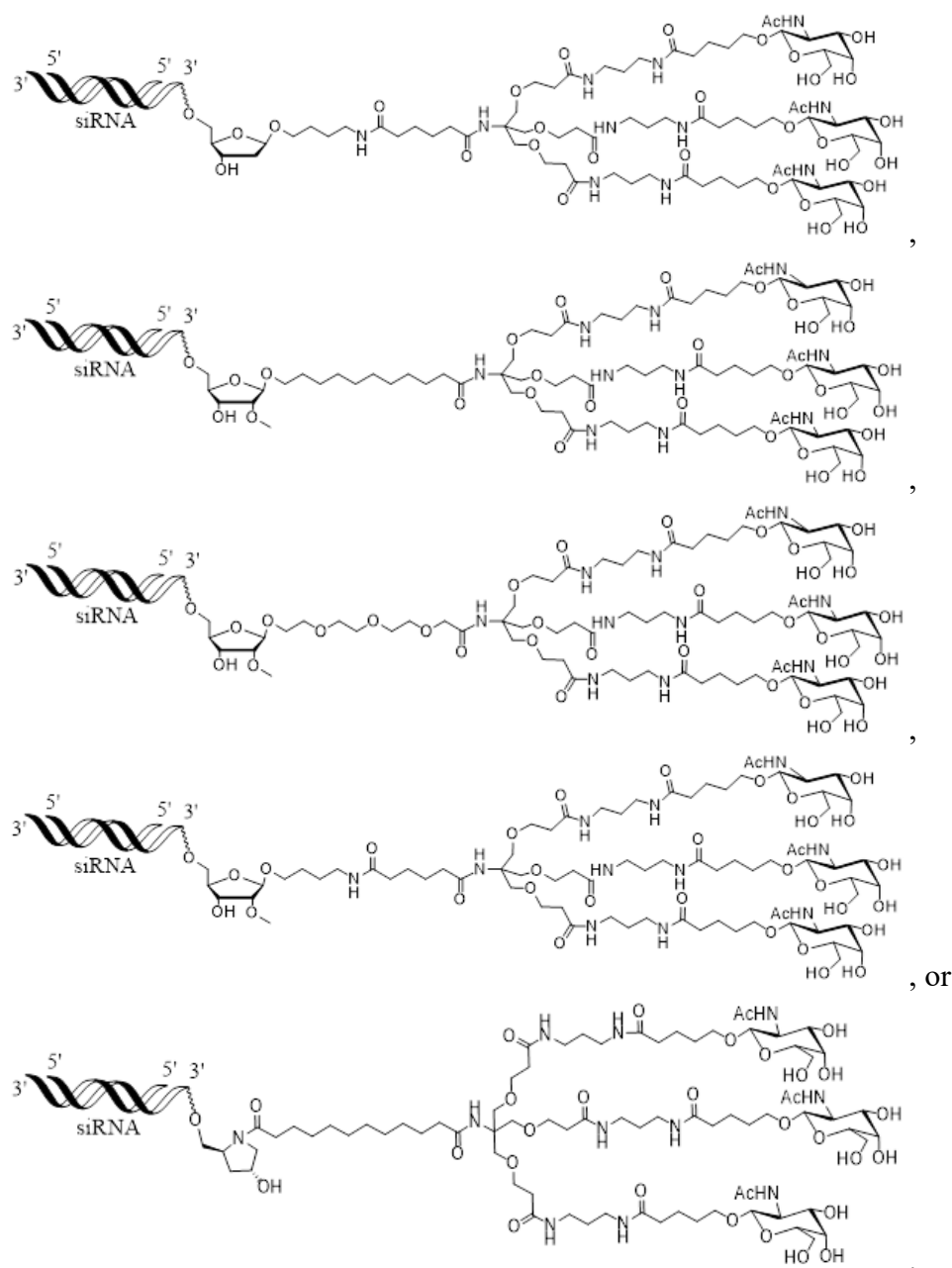
m is an integer from 0 to 4;

n is an integer from 0 to 4.

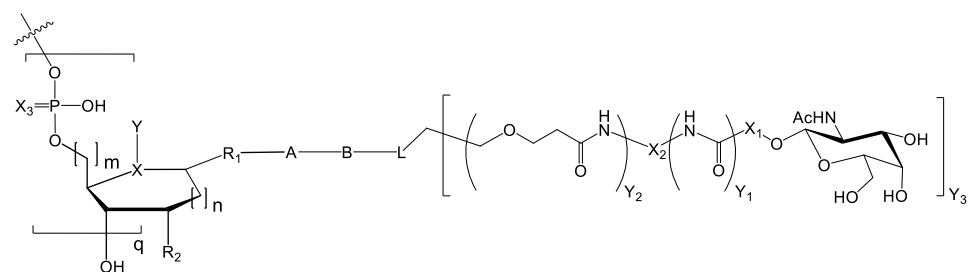
28. The conjugate according to claim 27, wherein the ligand is G4, G5, G6, or G7:



29. The conjugate according to claim 28, wherein the conjugate is represented by the following structures:



30. The conjugate according to claim 22, wherein the ligand is:



wherein X is oxygen, nitrogen, or sulfur;

Y is an alkyl or aromatic group;

R_1 is oxygen or sulfur;

R_2 is hydrogen, an amino group, a C_{1-4} alkyl group, an aromatic group, a C_{1-4} alkoxy group, or a halogen;

A is $-(CH_2)_a-$, $-(CH_2CH_2O)_b-$, $-((CH_2)_cNHCO)_d-$, or $-((CH_2)_cCONH)_d-$, where a is an integer from 1 to 15, b is an integer from 1 to 7, c is an integer from 1 to 7, and d is an integer from 1 to 5;

B is $-(CH_2)_e-$, where e is an integer from 0 to 7;

L is $-CONH-$ or $-NHCO-$;

X_1 is $-(CH_2)_f-$ or $-(CH_2CH_2O)_fCH_2-$, where f is an integer from 1 to 5;

X_2 is $-(CH_2)_g-$, where g is an integer from 1 to 6;

Y_1 is 0 or 1;

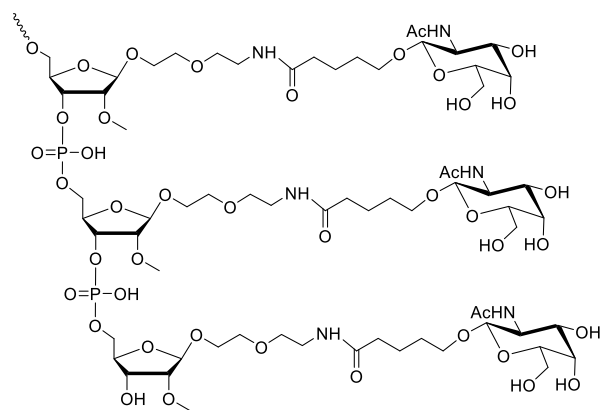
Y_2 is 0, 1, or 2;

Y_3 is 1, 2, or 3;

m is an integer from 0 to 4;

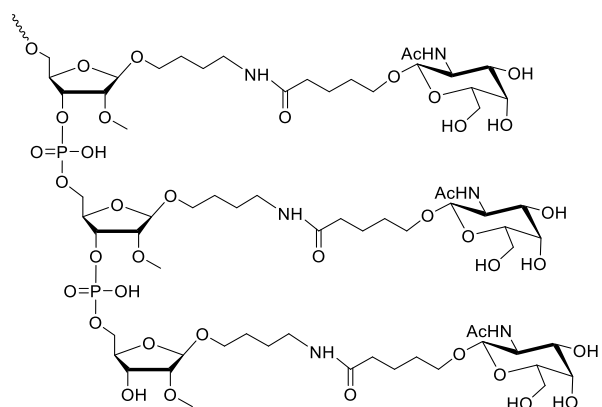
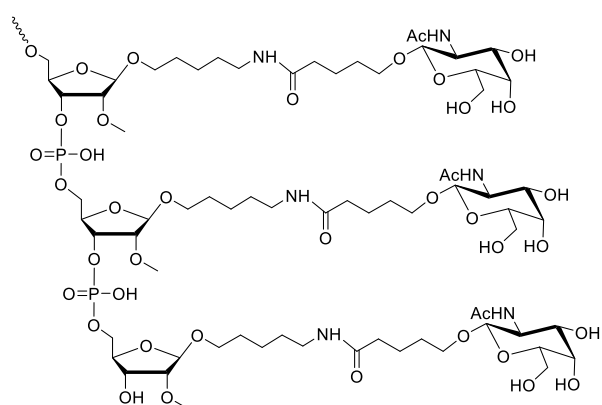
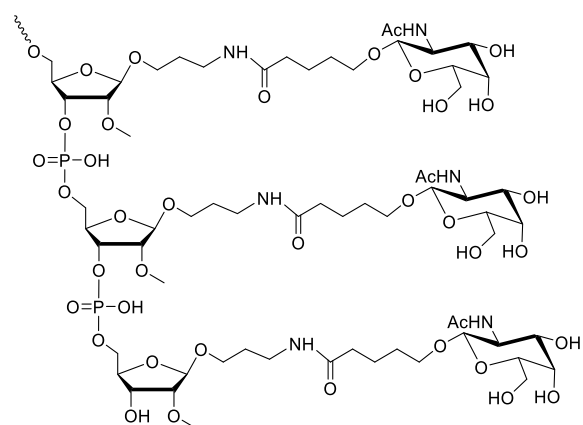
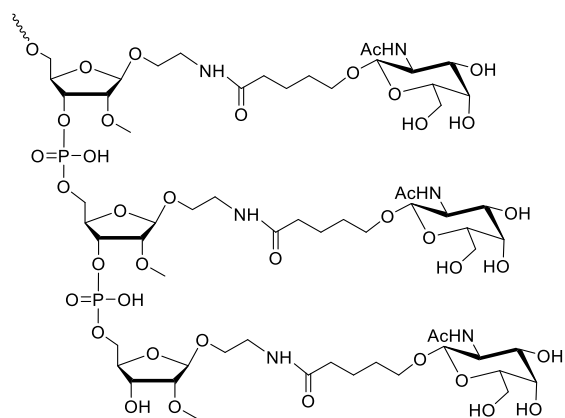
n is an integer from 0 to 4.

31. The conjugate according to claim 30, wherein the ligand is G101, G102, G103, G105, or G106:

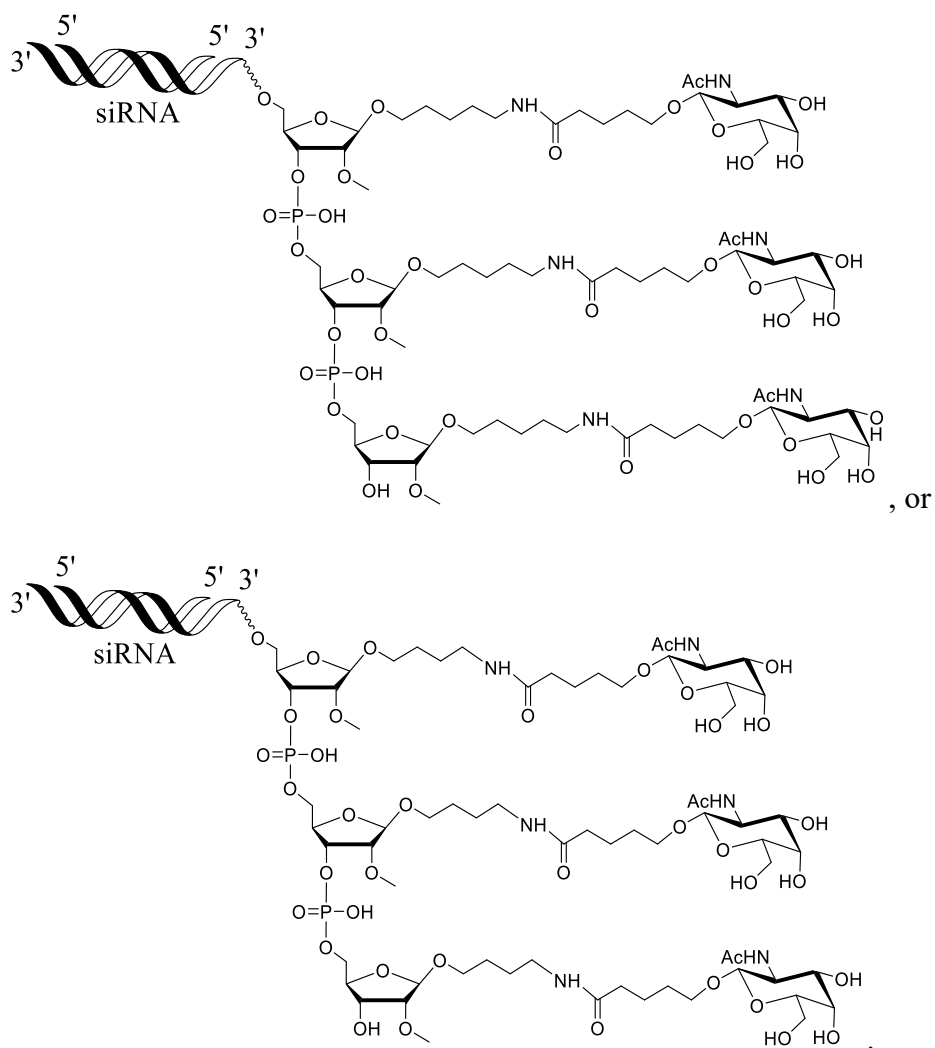


G101

,



32. The conjugate according to claim 31, wherein the conjugate is represented by the following structures:



33. The conjugate according to claim 22, wherein the conjugate comprises an oligonucleotide duplex selected from any one of the following sense and antisense strand pairs:

- (1) the sense strand has the sequence as shown in SEQ ID NO: 326, 327 or 328, and the antisense strand has the sequence as shown in SEQ ID NO: 179;
- (2) the sense strand has the sequence as shown in SEQ ID NO: 329, 330 or 331, and the antisense strand has the sequence as shown in SEQ ID NO: 181;
- (3) the sense strand has the sequence as shown in SEQ ID NO: 338 or 341, and the antisense strand has the sequence as shown in SEQ ID NO: 201;
- (4) the sense strand has the sequence as shown in SEQ ID NO: 350, 351 or 352, and the antisense strand has the sequence as shown in SEQ ID NO: 321.

34. The conjugate according to any one of claims 22 to 33, wherein the conjugate has the

function of inhibiting HBV gene expression.

35. A pharmaceutical composition comprising the double-stranded RNAi agent according to any one of claims 1 to 21 or the conjugate according to any one of claims 22 to 34, along with a pharmaceutically acceptable carrier.

36. The pharmaceutical composition according to claim 35, wherein the double-stranded RNAi agent or the conjugate is administered in a non-buffered solution.

37. The pharmaceutical composition according to claim 36, wherein the non-buffered solution is saline or water.

38. The pharmaceutical composition according to claim 35, wherein the double-stranded RNAi agent or the conjugate is administered with a buffered solution.

39. The pharmaceutical composition according to claim 38, wherein the buffered solution comprises acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof.

40. The pharmaceutical composition according to claim 39, wherein the buffered solution is phosphate-buffered solution.

41. The pharmaceutical composition according to claim 38, wherein the double-stranded RNAi agent or the conjugate is formulated into a lipid formulation for delivery within membranous molecular assemblies.

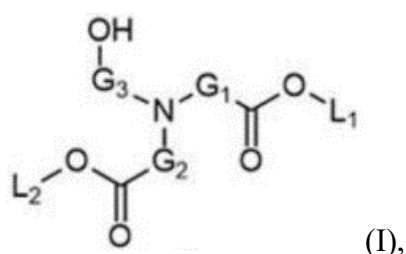
42. The pharmaceutical composition according to claim 41, wherein the lipid formulation is a nucleic acid-lipid particle.

43. The pharmaceutical composition according to claim 42, wherein the lipid formulation is a lipid nanoparticle.

44. The pharmaceutical composition according to claim 41, wherein a mass-to-mass ratio of lipid to the double-stranded RNAi agent or the conjugate is 1:1 to 50:1, 1:1 to 25:1, 3:1 to 15:1, 4:1 to 10:1, 5:1 to 9:1, or 6:1 to 9:1.

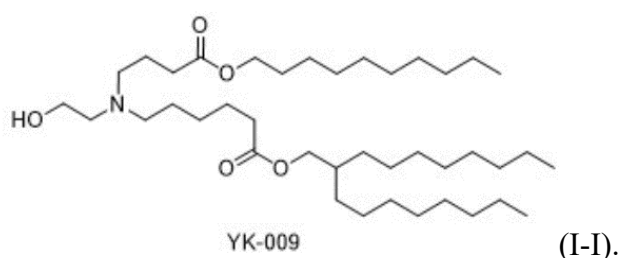
45. The pharmaceutical composition according to claim 43, wherein the lipid nanoparticle comprises a cationic lipid, a neutral lipid, a structural lipid, and a polymer-conjugated lipid.

46. The pharmaceutical composition according to claim 45, wherein the cationic lipid is a compound of formula (I), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,

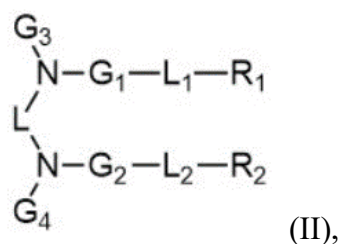


wherein G_1 is a C_{1-6} alkylene group; G_2 is a C_{2-8} alkylene group; G_3 is a C_{1-3} alkylene group; L_1 is a C_{6-15} linear alkyl group; L_2 is a C_{12-25} branched alkyl group.

47. The pharmaceutical composition according to claim 46, wherein the cationic lipid is YK-009 of formula (I-I):

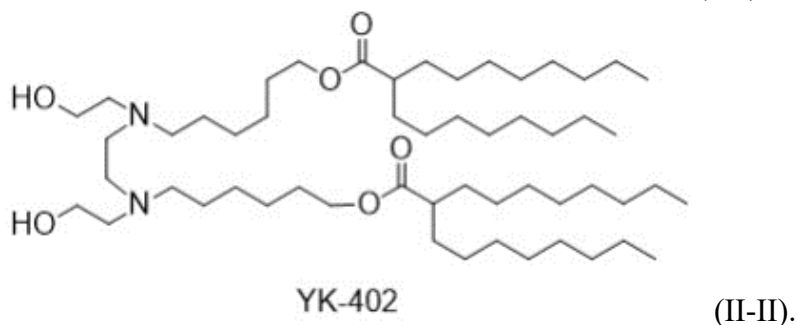
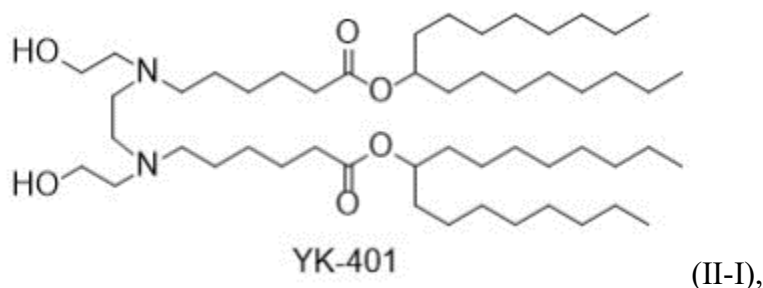


48. The pharmaceutical composition according to claim 45, wherein the cationic lipid is a compound of formula (II), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,

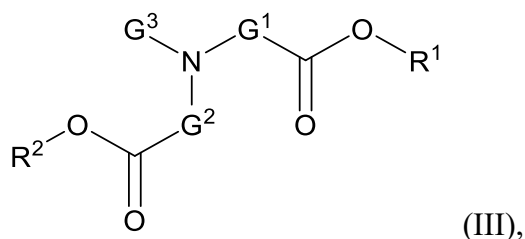


wherein G_1 is a C_{2-8} alkylene group; G_2 is a C_{2-8} alkylene group; L_1 is $-C(O)O-$ or $-OC(O)-$; L_2 is $-C(O)O-$ or $-OC(O)-$; R_1 is a C_{6-25} linear or branched alkyl group; R_2 is a C_{6-25} linear or branched alkyl group; G_3 is $HO(CH_2)_2-$ or $HO(CH_2)_3-$; G_4 is $HO(CH_2)_2-$ or $HO(CH_2)_3-$; L is $-(CH_2)_2-$, $-(CH_2)_3-$, or $-(CH_2)_4-$.

49. The pharmaceutical composition according to claim 48, wherein the cationic lipid is YK-401 of formula (II-I) or YK-402 of formula (II-II):



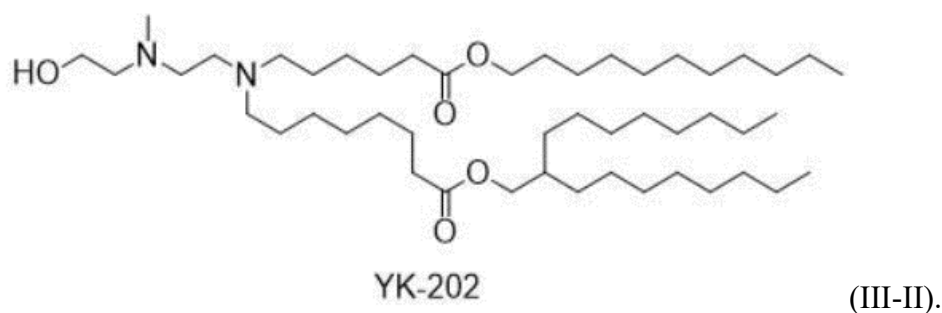
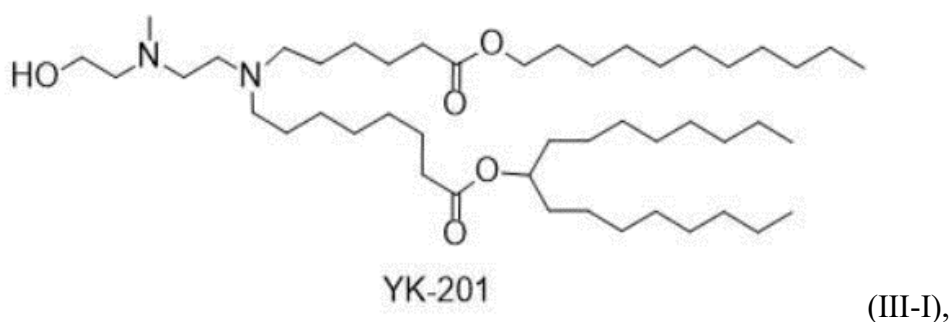
50. The pharmaceutical composition according to claim 45, wherein the cationic lipid is a compound of formula (III), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,



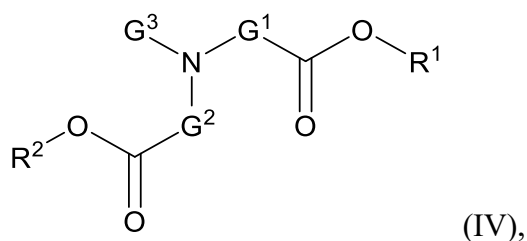
wherein G_1 is a C_{1-6} alkylene group; G_2 is a C_{2-8} alkylene group; R_1 is a C_{6-20} linear or

branched alkyl group; R_2 is a C_{12-25} branched alkyl group; G_3 is $HO(CH_2)_2N(CH_3)(CH_2)_2-$, $HO(CH_2)_2N(CH_2CH_3)(CH_2)_2-$, $(HO(CH_2)_2)_2N(CH_2)_2-$, $CH_3O(CH_2)_2N(CH_3)(CH_2)_2-$, $(CH_3)_2N(CH_2)_3SC(O)O(CH_2)_2-$, $(CH_3)_2N(CH_2)_3SC(O)-$, $CH_3NH(CH_2)_2N(CH_3)(CH_2)_2-$, or $CH_3CH_2NH(CH_2)_2-$.

51. The pharmaceutical composition according to claim 50, wherein the cationic lipid is YK-201 of formula (III-I) or YK-202 of formula (III-II):



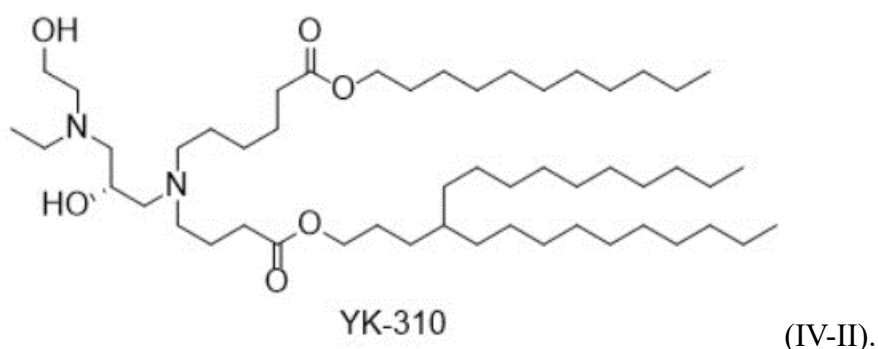
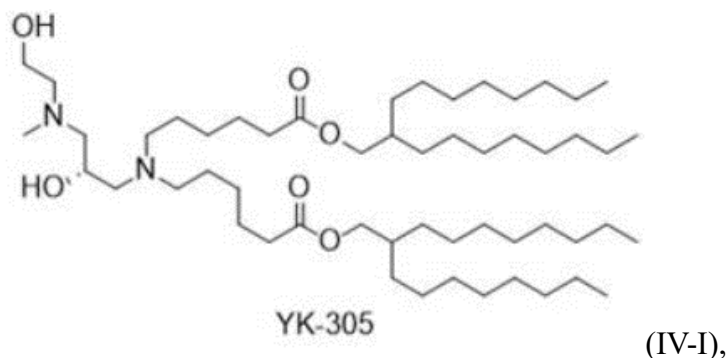
52. The pharmaceutical composition according to claim 45, wherein the cationic lipid is a compound of formula (IV), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,



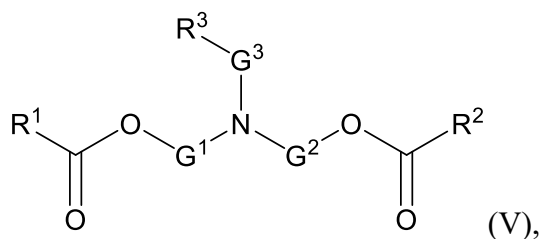
wherein G_1 is a C_{1-8} alkylene group; G_2 is a C_{2-8} alkylene group; R_1 is a C_{6-25} linear or branched alkyl group; R_2 is a C_{12-25} linear or branched alkyl group; G_3 is $HO(CH_2)_2N(R_3)CH_2CH(OH)CH_2-$, where R_3 is $-CH_3$, $-CH_2CH_3$, or $-CH_2CH_2OH$.

53. The pharmaceutical composition according to claim 52, wherein the cationic lipid is

YK-305 of formula (IV-I) or YK-310 of formula (IV-II):

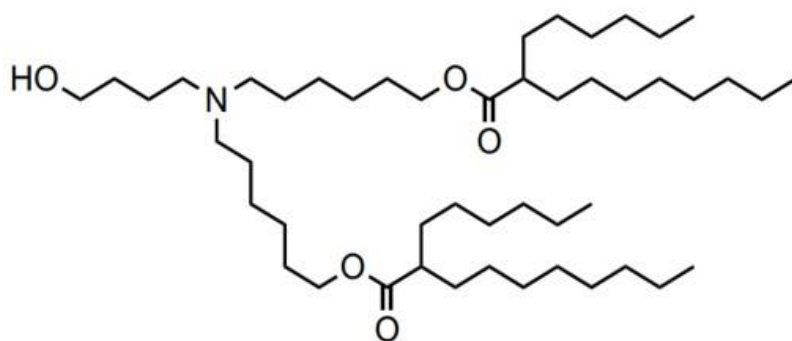


54. The pharmaceutical composition according to claim 45, wherein the cationic lipid is a compound of formula (V), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,



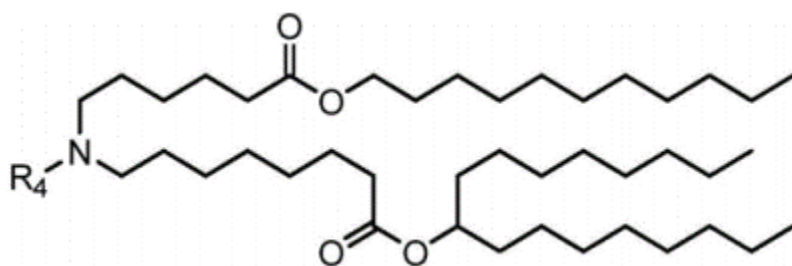
G^1 and G^2 are each independently an unsubstituted C_6 – C_{10} alkylene group; G^3 is an unsubstituted C_1 – C_{12} alkylene group; R^1 and R^2 are each independently a C_6 – C_{24} alkyl group or a C_6 – C_{24} alkenyl group; R^3 is OR^5 , N, $-C(=O)OR^4$, $-OC(=O)R^4$, or $-NR^5C(=O)R^4$; R^4 is a C_1 – C_{12} hydrocarbon group; and R^5 is H or a C_1 – C_6 hydrocarbon group.

55. The pharmaceutical composition according to claim 54, wherein the cationic lipid is ALC0315 of formula (V-I):



(V-I).

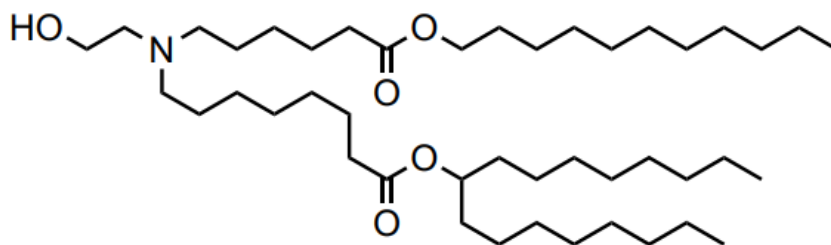
56. The pharmaceutical composition according to claim 45, wherein the cationic lipid is a compound of formula (VI), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,



(VI),

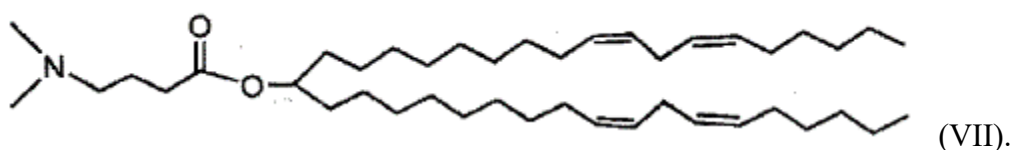
R₄ is selected from $-(CH_2)_nQ$ and $-(CH_2)_nCHQR$; Q is selected from the group consisting of -OR, -OH, $-O(CH_2)_nN(R)_2$, $-OC(O)R$, $-CX_3$, -CN, $-N(R)C(O)R$, $-N(H)C(O)R$, $-N(R)S(O)_2R$, $-N(H)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(H)C(O)N(R)_2$, $-N(H)C(O)N(H)(R)$, $-N(R)C(S)N(R)_2$, $-N(H)C(S)N(R)_2$, $-N(H)C(S)N(H)(R)$, $-N(R)S(O)_2R_8$, and a heterocyclic group; n is 1, 2, or 3.

57. The pharmaceutical composition according to claim 56, wherein the cationic lipid is SM102 of formula (VI-I):



(VI-I).

58. The pharmaceutical composition according to claim 45, wherein the cationic lipid is a compound DLIN-MC3-DMA of formula (VII), or a N-oxide, solvate, pharmaceutically acceptable salt, or stereoisomer thereof,



59. The pharmaceutical composition according to claim 45, wherein the cationic lipid comprises one or more selected from YK-009, YK-401, YK-305, ALC-0315, SM-102, and DLin-MC3-DMA.

60. The pharmaceutical composition according to claim 45, wherein the cationic lipid and the neutral lipid are present in a molar ratio of 1:1 to 10:1.

61. The pharmaceutical composition according to claim 45, wherein the cationic lipid and the structural lipid are present in a molar ratio of 1:1 to 5:1.

62. The pharmaceutical composition according to claim 45, wherein the cationic lipid, the neutral lipid, the structural lipid, and the polymer-conjugated lipid are present in a molar ratio of (25–65):(5–25):(25–70):(0.5–5).

63. The pharmaceutical composition according to claim 62, wherein the cationic lipid, the neutral lipid, the structural lipid, and the polymer-conjugated lipid are present in a molar ratio of (25–65):(5–25):(25–45):(0.5–5).

64. The pharmaceutical composition according to claim 63, wherein the cationic lipid, the neutral lipid, the structural lipid, and the polymer-conjugated lipid are present in a molar ratio of 50:10:38.5:1.5 or 49:10:39.5:1.5.

65. The pharmaceutical composition according to claim 45, wherein the neutral lipid comprises one or more selected from phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, ceramide, sterols, and derivatives thereof.

66. The pharmaceutical composition according to claim 65, wherein the neutral lipid is

selected from one or more of the following: 1,2-dilinoleoyl-sn-glycero-3-phosphocholine, 1,2-dimyristoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphocholine, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, 1,2-distearoyl-sn-glycero-3-phosphocholine, 1,2-diundecanoyl-sn-glycero-3-phosphocholine, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine, 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine, 1-hexadecyl-sn-glycero-3-phosphocholine, 1,2-dilinenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt, dipalmitoylphosphatidylglycerol, palmitoyl-oleoylphosphatidylethanolamine, distearoylphosphatidylethanolamine, dipalmitoylphosphatidylethanolamine, dimyristoylphosphatidylethanolamine, 1-stearoyl-2-oleoyl-stearoylethanolamine, 1-stearoyl-2-oleoyl-phosphatidylcholine, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyl-oleoylphosphatidylcholine, lysophosphatidylcholine, lysophosphatidylethanolamine, and mixtures thereof.

67. The pharmaceutical composition according to claim 66, wherein the neutral lipid is 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine and/or 1,2-distearoyl-sn-glycero-3-phosphocholine.

68. The pharmaceutical composition according to claim 45, wherein the structural lipid is selected from one or more of the following: cholesterol, non-sterols, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, α -tocopherol, and corticosteroids.

69. The pharmaceutical composition according to claim 68, wherein the structural lipid is

cholesterol.

70. The pharmaceutical composition according to claim 45, wherein the polymer-conjugated lipid is selected from one or more of the following: PEG-modified phosphatidylethanolamine, PEG-modified phosphatidic acid, PEG-modified ceramide, PEG-modified dialkylamine, PEG-modified diacylglycerol, and PEG-modified dialkylglycerol.

71. The pharmaceutical composition according to claim 70, wherein the polymer-conjugated lipid is selected from one or more of the following: distearoylphosphatidylethanolamine-polyethylene glycol 2000, dimyristoylglycerol-3-methoxypolyethylene glycol 2000, and methoxypolyethylene glycol bis(tetradecyl)acetamide.

72. A kit comprising a kit A, wherein the kit A comprises one or more of the double-stranded RNAi agent according to any one of claims 1 to 21, the conjugate according to any one of claims 22 to 34, or the pharmaceutical composition according to any one of claims 35 to 71.

73. The kit according to claim 72, wherein the kit further comprises a kit B, and the kit B comprises one or both of the following:

(1) an additional drug that reduces HBV gene expression or a composition comprising the drug that reduces HBV gene expression;

(2) one or more agents selected from the group consisting of hormone preparations, targeted small molecule agents, proteasome inhibitors, imaging agents, diagnostic agents, chemotherapeutic agents, oncolytic drugs, cytotoxic agents, cytokines, activators of co-stimulatory molecules, inhibitors of inhibitory molecules, and vaccines.

74. Use of the double-stranded RNAi agent according to any one of claims 1 to 21, the conjugate according to any one of claims 22 to 34, or the pharmaceutical composition according to any one of claims 35 to 71 in the preparation of a medicament for preventing and/or treating a disease associated with HBV gene expression.

75. The use according to claim 74, wherein the disease associated with HBV gene expression is selected from the following disease types: chronic hepatitis B, liver fibrosis, liver cirrhosis, liver cancer, acute hepatitis B, and HBV/hepatitis D virus co-infection-associated diseases.