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(54) **Title:**
MODULATION OF HEPATITIS B VIRUS (HBV) EXPRESSION

(57) **Abstract:**
Disclosed herein are antisense compounds and methods for decreasing HBV mRNA, DNA and protein expression. Such methods, compounds, and compositions are useful to treat, prevent, or ameliorate HBV-related diseases, disorders or conditions.

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MODULATION OF HEPATITIS B VIRUS (HBV) EXPRESSION

Sequence Listing

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0175WOSEQ.txt created April 18, 2012, which is approximately 256 KB in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

Field

In certain embodiments provided are methods, compounds, and compositions for inhibiting expression of hepatitis B virus (HBV) mRNA and protein in an animal. Such methods, compounds, and compositions are useful to treat, prevent, or ameliorate HBV-related diseases and disorders.

Background

Hepatitis B is a viral disease transmitted parenterally by contaminated material such as blood and blood products, contaminated needles, sexually and vertically from infected or carrier mothers to their offspring. It is estimated by the World Health Organization that more than 2 billion people have been infected worldwide, with about 4 million acute cases per year, 1 million deaths per year, and 350-400 million chronic carriers (World Health Organization: Geographic Prevalence of Hepatitis B Prevalence, 2004. http://www.who.int/vaccines-surveillance/graphics/htmls/hepbprev.htm).

The virus, HBV, is a double-stranded hepatotropic virus which infects only humans and non-human primates. Viral replication takes place predominantly in the liver and, to a lesser extent, in the kidneys, pancreas, bone marrow and spleen (Hepatitis B virus biology. Microbiol Mol Biol Rev. 64: 2000; 51-68.). Viral and immune markers are detectable in blood and characteristic antigen-antibody patterns evolve over time. The first detectable viral marker is HBsAg, followed by hepatitis B e antigen (HBeAg) and HBV DNA. Titers may be high during the incubation period, but HBV DNA and HBeAg levels begin to fall at the onset of illness and may be undetectable at the time of peak clinical illness (Hepatitis B virus infection—natural history and clinical consequences. N Engl J Med.. 350: 2004; 1118-1129). HBeAg is a viral marker detectable in blood and correlates with active viral replication, and therefore high viral load and infectivity (Hepatitis B e antigen—the dangerous end game of hepatitis B. N Engl J Med. 347: 2002; 208-210). The presence of anti-HBsAb and anti-HBcAb (IgG) indicates recovery and immunity in a previously infected

individual.

Currently the recommended therapies for chronic HBV infection by the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) include interferon alpha (INFα), pegylated interferon alpha-2a (Peg-IFN2a), entecavir, and tenofovir. The nucleoside and nucleotide therapies, entecavir and tenofovir, are successful at reducing viral load, but the rates of HBeAg seroconversion and HBsAg loss are even lower than those obtained using IFNα therapy. Other similar therapies, including lamivudine (3TC), telbivudine (LdT), and adefovir are also used, but for nucleoside/nucleotide therapies in general, the emergence of resistance limits therapeutic efficacy.

Thus, there is a need in the art to discover and develop new anti-viral therapies. Additionally, there is a need for new anti-HBV therapies capable of increasing HBeAg and HBsAg seroconversion rates. Recent clinical research has found a correlation between seroconversion and reductions in HBeAg (Fried et al (2008) Hepatology 47:428) and reductions in HBsAg (Moucari et al (2009) Hepatology 49:1151). Reductions in antigen levels may have allowed immunological control of HBV infection because high levels of antigens are thought to induce immunological tolerance. Current nucleoside therapies for HBV are capable of dramatic reductions in serum levels of HBV but have little impact on HBeAg and HBsAg levels.

Antisense technology is emerging as an effective means for reducing the expression of specific gene products and may therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications for the modulation of HBV expression (See U.S. Patent Publication Nos. 2008/0039418 and 2007/0299027). Antisense therapy differs from nucleoside therapy in that it can directly target the transcripts for the HBV antigens and thereby reduce serum HBeAg and HBsAg levels. Because of the multiple, overlapping transcripts produced upon HBV infection, there is also an opportunity for a single antisense oligomer to reduce HBV DNA in addition to both HBeAg and HBsAg. Therefore, antisense technology is emerging as an effective means for reducing the expression of certain gene products and may therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications for the modulation of HBV.

Summary

Provided herein are methods, compounds, and compositions for modulating expression of HBV mRNA and protein. In certain embodiments, compounds useful for modulating expression of HBV mRNA and protein are antisense compounds. In certain embodiments, the antisense compounds are antisense oligonucleotides.

In certain embodiments, modulation can occur in a cell or tissue. In certain embodiments, the cell or tissue is in an animal. In certain embodiments, the animal is a human. In certain embodiments, HBV

mRNA levels are reduced. In certain embodiments, HBV DNA levels are reduced. In certain embodiments, HBV protein levels are reduced. In certain embodiments, HBV antigen levels are reduced. In certain embodiments, HBV s-antigen (HBsAg) levels are reduced. In certain embodiments, HBV e-antigen (HBeAg) levels are reduced. Such reduction can occur in a time-dependent manner or in a dose-dependent manner.

Also provided are methods, compounds, and compositions useful for preventing, treating, and ameliorating diseases, disorders, and conditions. In certain embodiments, such HBV related diseases, disorders, and conditions are liver diseases. In certain embodiments, such liver diseases, disorders, and conditions includes jaundice, liver cancer, liver inflammation, liver fibrosis, inflammation, liver cirrhosis, liver failure, diffuse hepatocellular inflammatory disease, hemophagocytic syndrome, serum hepatitis, HBV viremia, and liver disease–related transplantation. In certain embodiments, such HBV related diseases, disorders, and conditions are hyperproliferative diseases, disorders, and conditions. In certain embodiments such hyperproliferative diseases, disorders, and conditions include cancer as well as associated malignancies and metastases. In certain embodiments, such cancers include liver cancer and hepatocellular cancer (HCC).

Such diseases, disorders, and conditions can have one or more risk factors, causes, or outcomes in common. Certain risk factors and causes for development of liver disease or a hyperproliferative disease include growing older; tobacco use; exposure to sunlight and ionizing radiation; contact with certain chemicals; infection with certain viruses and bacteria; certain hormone therapies; family history of cancer; alcohol use; and certain lifestyle choices including poor diet, lack of physical activity, and/or being overweight. Certain symptoms and outcomes associated with development of a liver disease or a hyperproliferative disease include but are not limited to: flu-like illness, weakness, aches, headache, fever, loss of appetite, diarrhea, jaundice, nausea and vomiting, pain over the liver area of the body, clay- or grey-colored stool, itching all over, and dark-colored urine.

In certain embodiments, methods of treatment include administering a HBV antisense compound to an individual in need thereof. In certain embodiments, methods of treatment include administering a HBV antisense oligonucleotide to an individual in need thereof.

Detailed Description

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including" as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including, but not limited to, patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

Definitions

Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical synthesis, and chemical analysis. Where permitted, all patents, applications, published applications and other publications, GENBANK Accession Numbers and associated sequence information obtainable through databases such as National Center for Biotechnology Information (NCBI) and other data referred to throughout in the disclosure herein are incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

Unless otherwise indicated, the following terms have the following meanings:

"2'-O-methoxyethyl" (also 2'-MOE and 2'-O$(CH_2)_2$-OCH_3) refers to an O-methoxy-ethyl modification at the 2' position of a furanose ring. A 2'-O-methoxyethyl modified sugar is a modified sugar.

"2'-MOE nucleoside" (also 2'-O-methoxyethyl nucleoside) means a nucleoside comprising a 2'-MOE modified sugar moiety.

"2'-substituted nucleoside" means a nucleoside comprising a substituent at the 2'-position of the furanosyl ring other than H or OH. In certain embodiments, 2' substituted nucleosides include nucleosides with bicyclic sugar modifications.

"3' target site" refers to the nucleotide of a target nucleic acid which is complementary to the 3'-most nucleotide of a particular antisense compound.

"5' target site" refers to the nucleotide of a target nucleic acid which is complementary to the 5'-most nucleotide of a particular antisense compound.

"5-methylcytosine" means a cytosine modified with a methyl group attached to the 5 position. A 5-methylcytosine is a modified nucleobase.

"About" means within ±7% of a value. For example, if it is stated, "the compounds affected at least about 70% inhibition of HBV", it is implied that the HBV levels are inhibited within a range of 63% and 77%.

"Acceptable safety profile" means a pattern of side effects that is within clinically acceptable limits.

"Active pharmaceutical agent" means the substance or substances in a pharmaceutical composition that provide a therapeutic benefit when administered to an individual. For example, in certain embodiments an antisense oligonucleotide targeted to HBV is an active pharmaceutical agent.

"Active target region" means a target region to which one or more active antisense compounds is targeted. "Active antisense compounds" means antisense compounds that reduce target nucleic acid levels or protein levels.

"Acute hepatitis B infection" results when a person exposed to the hepatitis B virus begins to develop the signs and symptoms of viral hepatitis. This period of time, called the incubation period, is an average of 90 days, but could be as short as 45 days or as long as 6 months. For most people this infection will cause mild to moderate discomfort but will go away by itself because of the body's immune response succeeds in fighting the virus. However, some people, particularly those with compromised immune systems, such as persons suffering from AIDS, undergoing chemotherapy, taking immunosuppressant drugs, or taking steroids, have very serious problems as a result of the acute HBV infection, and go on to more severe conditions such as fulminant liver failure.

"Administered concomitantly" refers to the co-administration of two agents in any manner in which the pharmacological effects of both are manifest in the patient at the same time. Concomitant administration does not require that both agents be administered in a single pharmaceutical composition, in the same dosage form, or by the same route of administration. The effects of both agents need not manifest themselves at the same time. The effects need only be overlapping for a period of time and need not be coextensive.

"Administering" means providing a pharmaceutical agent to an individual, and includes, but is not limited to administering by a medical professional and self-administering.

"Agent" means an active substance that can provide a therapeutic benefit when administered to an animal. "First Agent" means a therapeutic compound described herein. For example, a first agent can be an antisense oligonucleotide targeting HBV. "Second agent" means a second therapeutic compound described herein (e.g. a second antisense oligonucleotide targeting HBV) and/or a non- HBV therapeutic compound.

"Amelioration" refers to a lessening of at least one indicator of the severity of a condition or disease. The severity of indicators may be determined by subjective or objective measures which are known to those skilled in the art.

"Animal" refers to a human or non-human animal, including, but not limited to, mice, rats, rabbits, dogs, cats, pigs, and non-human primates, including, but not limited to, monkeys and chimpanzees.

"Antibody" refers to a molecule characterized by reacting specifically with an antigen in some way, where the antibody and the antigen are each defined in terms of the other. Antibody may refer to a complete antibody molecule or any fragment or region thereof, such as the heavy chain, the light chain, F_{ab} region, and F_c region.

"Antisense activity" means any detectable or measurable activity attributable to the hybridization of an antisense compound to its target nucleic acid. In certain embodiments, antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid.

"Antisense compound" means an oligomeric compound that is is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding. Examples of antisense compounds include single-stranded and double-stranded compounds, such as, antisense oligonucleotides, siRNAs, shRNAs, snoRNAs, miRNAs, and satellite repeats.

"Antisense inhibition" means reduction of target nucleic acid levels in the presence of an antisense compound complementary to a target nucleic acid compared to target nucleic acid levels in the absence of the antisense compound.

"Antisense mechanisms" are all those mechanisms involving hybridization of a compound with target nucleic acid, wherein the outcome or effect of the hybridization is either target degradation or target occupancy with concomitant stalling of the cellular machinery involving, for example, transcription or splicing.

"Antisense oligonucleotide" means a single-stranded oligonucleotide having a nucleobase sequence that permits hybridization to a corresponding region or segment of a target nucleic acid.

"Base complementarity" refers to the capacity for the precise base pairing of nucleobases of an antisense oligonucleotide with corresponding nucleobases in a target nucleic acid (i.e., hybridization), and is mediated by Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen binding between corresponding nucleobases.

"Bicyclic sugar" means a furanose ring modified by the bridging of two non-geminal carbon atoms. A bicyclic sugar is a modified sugar.

"Body weight" refers to an animal's whole body weight, inclusive of all tissues including adipose tissue.

"Cap structure" or "terminal cap moiety" means chemical modifications, which have been incorporated at either terminus of an antisense compound.

"cEt" or "constrained ethyl" means a bicyclic sugar moiety comprising a bridge connecting the 4'-carbon and the 2'-carbon, wherein the bridge has the formula: 4'-$CH(CH_3)$-O-2'.

"Constrained ethyl nucleoside" (also cEt nucleoside) means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-$CH(CH_3)$-O-2' bridge.

"Chemically distinct region" refers to a region of an antisense compound that is in some way chemically different than another region of the same antisense compound. For example, a region having 2'-O-methoxyethyl nucleotides is chemically distinct from a region having nucleotides without 2'-O-methoxyethyl modifications.

"Chimeric antisense compounds" means antisense compounds that have at least 2 chemically distinct regions, each position having a plurality of subunits.

"Chronic hepatitis B infection" occurs when a person initially suffers from an acute infection but is then unable to fight off the infection. Whether the disease becomes chronic or completely resolves depends mostly on the age of the infected person. About 90% of infants infected at birth will progress to chronic disease. However, as a person ages, the risk of chronic infection decreases such that between 20%-50% of children and less than 10% of older children or adults will progress from acute to chronic infection. Chronic HBV infections are the primary treatment goal for embodiments of the present invention, although ASO compositions of the present invention are also capable of treating HBV-related conditions, such as inflammation, fibrosis, cirrhosis, liver cancer, serum hepatitis, and more.

"Co-administration" means administration of two or more pharmaceutical agents to an individual. The two or more pharmaceutical agents may be in a single pharmaceutical composition, or may be in separate pharmaceutical compositions. Each of the two or more pharmaceutical agents may be administered through the same or different routes of administration. Co-administration encompasses administration in parallel or sequentially.

"Complementarity" means the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

"Comply" means the adherence with a recommended therapy by an individual.

"Comprise," "comprises" and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

"Contiguous nucleobases" means nucleobases immediately adjacent to each other.

"Cure" means a method or course that restores health or a prescribed treatment for an illness.

"Deoxyribonucleotide" means a nucleotide having a hydrogen at the 2' position of the sugar portion of the nucleotide. Deoxyribonucleotides may be modified with any of a variety of substituents.

"Designing" or "Designed to" refer to the process of designing an oligomeric compound that specifically hybridizes with a selected nucleic acid molecule.

"Diluent" means an ingredient in a composition that lacks pharmacological activity, but is pharmaceutically necessary or desirable. For example, in drugs that are injected, the diluent may be a liquid, e.g. saline solution.

"Dosage unit" means a form in which a pharmaceutical agent is provided, e.g. pill, tablet, or other dosage unit known in the art.

"Dose" means a specified quantity of a pharmaceutical agent provided in a single administration, or in a specified time period. In certain embodiments, a dose may be administered in two or more boluses, tablets, or injections. For example, in certain embodiments, where subcutaneous administration is desired, the desired dose requires a volume not easily accommodated by a single injection. In such embodiments, two or more injections may be used to achieve the desired dose. In certain embodiments, a dose may be administered in two or more injections to minimize injection site reaction in an individual. In other embodiments, the pharmaceutical agent is administered by infusion over an extended period of time or continuously. Doses may be stated as the amount of pharmaceutical agent per hour, day, week or month.

"Dosing regimen" is a combination of doses designed to achieve one or more desired effects.

"Duration" means the period of time during which an activity or event continues. In certain embodiments, the duration of treatment is the period of time during which doses of a pharmaceutical agent are administered.

"Effective amount" in the context of modulating an activity or of treating or preventing a condition means the administration of that amount of active ingredient to a subject in need of such modulation, treatment or prophylaxis, either in a single dose or as part of a series, that is effective for modulation of that

effect, or for treatment or prophylaxis or improvement of that condition. The effective amount will vary depending upon the health and physical condition of the subject to be treated, the taxonomic group of subjects to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors.

"Efficacy" means the ability to produce a desired effect.

"Expression" includes all the functions by which a gene's coded information is converted into structures present and operating in a cell. Such structures include, but are not limited to the products of transcription and translation.

"Fully complementary" or "100% complementary" means each nucleobase of a first nucleic acid has a complementary nucleobase in a second nucleic acid. In certain embodiments, a first nucleic acid is an antisense compound and a target nucleic acid is a second nucleic acid.

"Fully modified motif" refers to an antisense compound comprising a contiguous sequence of nucleosides wherein essentially each nucleoside is a sugar modified nucleoside having uniform modification.

"Gapmer" means a chimeric antisense compound in which an internal region having a plurality of nucleosides that support RNase H cleavage is positioned between external regions having one or more nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from the nucleoside or nucleosides comprising the external regions. The internal region may be referred to as the "gap" and the external regions may be referred to as the "wings."

"Gap-widened" means an antisense compound having a gap segment of 12 or more contiguous 2'-deoxyribonucleotides positioned between 5' and 3' wing segments having from one to six nucleotides having modified sugar moieties.

"HBV" means mammalian hepatitis B virus, including human hepatitis B virus. The term encompasses geographical genotypes of hepatitis B virus, particularly human hepatitis B virus, as well as variant strains of geographical genotypes of hepatitis B virus.

"HBV antigen" means any hepatitis B virus antigen or protein, including core proteins such as "hepatitis B core antigen" or "HBcAG" and "hepatitis B E antigen" or "HBeAG" and envelope proteins such as "HBV surface antigen", or "HBsAg" or "HBsAG".

"Hepatitis B E antigen" or "HBeAg" or "HBeAG" is a secreted, non-particulate form of HBV core protein. HBV antigens HBeAg and HBcAg share primary amino acid sequences, so show cross-reactivity at the T cell level. HBeAg is not required for viral assembly or replication, although studies suggest they may

be required for establishment of chronic infection. Neonatal infection with HBeAg-negative mutant often results in fulminant acute rather than chronic HBV infection (Terezawa et al (1991) Pediatr. Res. 29:5), whereas infection of young woodchucks with WHeAg-negative mutant results in a much lower rate of chronic WHV infection (Cote et al (2000) Hepatology 31:190). HBeAg may possibly function as a toleragen by inactivating core specific T cells through deletion or clonal anergy (Milich et al (1998) J. Immunol. 160:8102). There is a positive correlation between reduction of HBV viral load and antigens, and a decrease of expression, by T cells, of the inhibitory receptor programmed death-1 (PD-1; also known as PDCD1), a negative regulator of activated T cells, upon antiviral therapy and HBeAg seroconversion (Evans et al (2008) Hepatology 48:759).

"HBV mRNA" means any messenger RNA expressed by hepatitis B virus.

"HBV nucleic acid" or 'HBV DNA" means any nucleic acid encoding HBV. For example, in certain embodiments, a HBV nucleic acid includes, without limitation, any viral DNA sequence encoding a HBV genome or portion thereof, any RNA sequence transcribed from a viral DNA including any mRNA sequence encoding a HBV protein.

"HBV protein" means any protein secreted by hepatitis B virus The term encompasses various HBV antigens, including core proteins such as "Hepatitis E antigen", "HBeAg" or "HBeAG" and envelope proteins such as "HBV surface antigen", or "HBsAg".

"HBV surface antigen", or "HBsAg", or "HBsAG" is the envelope protein of infectious HBV viral particles but is also secreted as a non-infectious particle with serum levels 1000-fold higher than HBV viral particles. The serum levels of HBsAg in an infected person or animal can be as high as 1000 μg/mL (Kann and Gehrlich (1998) Topley & Wilson's Microbiology and Microbial Infections, 9th ed. 745). In acute HBV infections, the half-life of HBsAg in the serum, or serum $t_{½}$, is 8.3 days (Chulanov et al (2003) J. Med. Virol. 69: 313). Internalization of HBsAg by myeloid dendritic cells inhibits up-regulation of co-stimulatory molecules (i.e. B7) and inhibits T cell stimulatory capacity (den Brouw et al (2008) Immunology 126:280), and dendritic cells from chronically infected patients also show deficits in expression of co-stimulatory molecules, secretion of IL-12, and stimulation of T cells in the presence of HBsAg (Zheng et al (2004) J. Viral Hepatitis 11:217). HBsAg specific CD8 cells from CHB patients show altered tetramer binding. These CD8 cells are not anergic but may have TCR topology that confers partial tolerance or ignorance (Reignat et al (2002) J. Exp. Med. 195:1089). Moreover, reduction in serum HBsAg > 1 log at week 24 has a high predictive value (92%) for sustained virological response (SVR – defined as nondetectable HBV DNA by PCR at 1 year after treatment) during Peg-IFNα2a therapy (Moucari et al (2009) Hepatology 49:1151).

"Hepatitis B-related condition" or "HBV-related condition" means any disease, biological condition, medical condition, or event which is exacerbated, caused by, related to, associated with, or traceable to a hepatitis B infection, exposure, or illness. The term hepatitis B-related condition includes chronic HBV

infection, inflammation, fibrosis, cirrhosis, liver cancer, serum hepatitis, jaundice, liver cancer, liver inflammation, liver fibrosis, liver cirrhosis, liver failure, diffuse hepatocellular inflammatory disease, hemophagocytic syndrome, serum hepatitis, HBV viremia, liver disease related to transplantation, and conditions having symptoms which may include any or all of the following: flu-like illness, weakness, aches, headache, fever, loss of appetite, diarrhea, nausea and vomiting, pain over the liver area of the body, clay- or grey-colored stool, itching all over, and dark-colored urine, when coupled with a positive test for presence of a hepatitis B virus, a hepatitis B viral antigen, or a positive test for the presence of an antibody specific for a hepatitis B viral antigen..

"Hybridization" means the annealing of complementary nucleic acid molecules. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an antisense compound and a nucleic acid target. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an antisense oligonucleotide and a nucleic acid target.

"Identifying an animal having an HBV infection" means identifying an animal having been diagnosed with an HBV; or, identifying an animal having any symptom of an HBV infection including, but not limited to chronic HBV infection, inflammation, fibrosis, cirrhosis, liver cancer, serum hepatitis, jaundice, liver cancer, liver inflammation, liver fibrosis, liver cirrhosis, liver failure, diffuse hepatocellular inflammatory disease, hemophagocytic syndrome, serum hepatitis, HBV viremia, liver disease related to transplantation, and conditions having symptoms which may include any or all of the following: flu-like illness, weakness, aches, headache, fever, loss of appetite, diarrhea, nausea and vomiting, pain over the liver area of the body, clay- or grey-colored stool, itching all over, and dark-colored urine, when coupled with a positive test for presence of a hepatitis B virus, a hepatitis B viral antigen, or a positive test for the presence of an antibody specific for a hepatitis B viral antigen, when coupled with a positive test for presence of a hepatitis B virus, a hepatitis B viral antigen, or a positive test for the presence of an antibody specific for a hepatitis B viral antigen.

"Immediately adjacent" means there are no intervening elements between the immediately adjacent elements.

"Individual" means a human or non-human animal selected for treatment or therapy.

"Individual compliance" means adherence to a recommended or prescribed therapy by an individual.

"Induce", "inhibit", "potentiate", "elevate", "increase", "decrease" or the like, generally denote quantitative differences between two states. Such terms may refer to a statistically significant difference between the two states. For example, "an amount effective to inhibit the activity or expression of HBV"

means that the level of activity or expression of HBV in a treated sample will quantitatively differ, and may be statistically significant, from the level of HBV activity or expression in untreated cells. Such terms are applied to, for example, levels of expression, and levels of activity.

"Inhibiting HBV" means reducing the level or expression of an HBV mRNA, DNA and/or protein. In certain embodiments, HBV is inhibited in the presence of an antisense compound targeting HBV, including an antisense oligonucleotide targeting HBV, as compared to expression of HBV mRNA, DNA and/or protein levels in the absence of a HBV antisense compound, such as an antisense oligonucleotide.

"Inhibiting the expression or activity" refers to a reduction, blockade of the expression or activity and does not necessarily indicate a total elimination of expression or activity.

"Injection site reaction" means inflammation or abnormal redness of skin at a site of injection in an individual.

"Internucleoside linkage" refers to the chemical bond between nucleosides.

"Intraperitoneal administration" means administration through infusion or injection into the peritoneum.

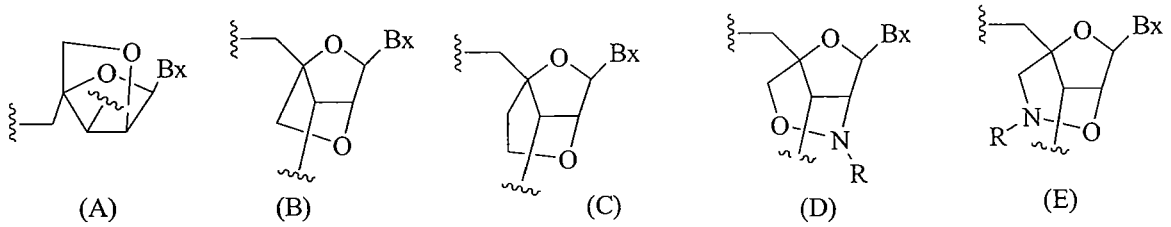
"Intravenous administration" means administration into a vein.

"Lengthened" antisense oligonucleotides are those that have one or more additional nucleosides relative to an antisense oligonucleotide disclosed herein.

"Linked deoxynucleoside" means a nucleic acid base (A, G, C, T, U) substituted by deoxyribose linked by a phosphate ester to form a nucleotide.

"Linked nucleosides" means adjacent nucleosides linked together by an internucleoside linkage.

"Locked nucleic acid" or " LNA" or "LNA nucleosides" means nucleic acid monomers having a bridge connecting two carbon atoms between the 4' and 2'position of the nucleoside sugar unit, thereby forming a bicyclic sugar. Examples of such bicyclic sugar include, but are not limited to A) α-L-Methyleneoxy (4'-CH_2-O-2') LNA , (B) β-D-Methyleneoxy (4'-CH_2-O-2') LNA , (C) Ethyleneoxy (4'-$(CH_2)_2$-O-2') LNA , (D) Aminooxy (4'-CH_2-O-N(R)-2') LNA and (E) Oxyamino (4'-CH_2-N(R)-O-2') LNA, as depicted below.



(A) (B) (C) (D) (E)

As used herein, LNA compounds include, but are not limited to, compounds having at least one bridge between the 4' and the 2' position of the sugar wherein each of the bridges independently comprises 1 or from 2 to 4 linked groups independently selected from $-[C(R_1)(R_2)]_n-$, $-C(R_1)=C(R_2)-$, $-C(R_1)=N-$, $-C(=NR_1)-$, $-C(=O)-$, $-C(=S)-$, $-O-$, $-Si(R_1)_2-$, $-S(=O)_x-$ and $-N(R_1)-$; wherein: x is 0, 1, or 2; n is 1, 2, 3, or 4; each R_1 and R_2 is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, a heterocycle radical, a substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5-C_7 alicyclic radical, substituted C_5-C_7 alicyclic radical, halogen, OJ_1, NJ_1J_2, SJ_1, N_3, $COOJ_1$, acyl (C(=O)-H), substituted acyl, CN, sulfonyl ($S(=O)_2-J_1$), or sulfoxyl ($S(=O)-J_1$); and each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1-C_{12} aminoalkyl, substituted C_1-C_{12} aminoalkyl or a protecting group.

Examples of 4'- 2' bridging groups encompassed within the definition of LNA include, but are not limited to one of formulae: $-[C(R_1)(R_2)]_n-$, $-[C(R_1)(R_2)]_n-O-$, $-C(R_1R_2)-N(R_1)-O-$ or $-C(R_1R_2)-O-N(R_1)-$. Furthermore, other bridging groups encompassed with the definition of LNA are 4'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-$(CH_2)_3$-2', 4'-CH_2-O-2', 4'-$(CH_2)_2$-O-2', 4'-CH_2-O-$N(R_1)$-2' and 4'-CH_2-$N(R_1)$-O-2'- bridges, wherein each R_1 and R_2 is, independently, H, a protecting group or C_1-C_{12} alkyl.

Also included within the definition of LNA according to the invention are LNAs in which the 2'-hydroxyl group of the ribosyl sugar ring is connected to the 4' carbon atom of the sugar ring, thereby forming a methyleneoxy (4'-CH_2-O-2') bridge to form the bicyclic sugar moiety. The bridge can also be a methylene (-CH_2-) group connecting the 2' oxygen atom and the 4' carbon atom, for which the term methyleneoxy (4'-CH_2-O-2') LNA is used. Furthermore; in the case of the bicylic sugar moiety having an ethylene bridging group in this position, the term ethyleneoxy (4'-CH_2CH_2-O-2') LNA is used. α -L- methyleneoxy (4'-CH_2-O-2'), an isomer of methyleneoxy (4'-CH_2-O-2') LNA is also encompassed within the definition of LNA, as used herein.

"Mismatch" or "non-complementary nucleobase" refers to the case when a nucleobase of a first nucleic acid is not capable of pairing with the corresponding nucleobase of a second or target nucleic acid.

"Modified internucleoside linkage" refers to a substitution or any change from a naturally occurring internucleoside bond (i.e. a phosphodiester internucleoside bond).

"Modified nucleobase" means any nucleobase other than adenine, cytosine, guanine, thymidine, or uracil. An "unmodified nucleobase" means the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U).

"Modified nucleoside" means a nucleoside having, independently, a modified sugar moiety and/or modified nucleobase.

"Modified nucleotide" means a nucleotide having, independently, a modified sugar moiety, modified internucleoside linkage, or modified nucleobase.

"Modified oligonucleotide" means an oligonucleotide comprising at least one modified internucleoside linkage, a modified sugar, and/or a modified nucleobase.

"Modified sugar" means substitution and/or any change from a natural sugar moiety.

"Monomer" refers to a single unit of an oligomer. Monomers include, but are not limited to, nucleosides and nucleotides, whether naturally occuring or modified.

"Motif" means the pattern of unmodified and modified nucleosides in an antisense compound.

"Natural sugar moiety" means a sugar moiety found in DNA (2'-H) or RNA (2'-OH).

"Naturally occurring internucleoside linkage" means a 3' to 5' phosphodiester linkage.

"Non-complementary nucleobase" refers to a pair of nucleobases that do not form hydrogen bonds with one another or otherwise support hybridization.

"Nucleic acid" refers to molecules composed of monomeric nucleotides. A nucleic acid includes, but is not limited to, ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single-stranded nucleic acids, double-stranded nucleic acids, small interfering ribonucleic acids (siRNA), and microRNAs (miRNA).

"Nucleobase" means a heterocyclic moiety capable of pairing with a base of another nucleic acid.

"Nucleobase complementarity" refers to a nucleobase that is capable of base pairing with another nucleobase. For example, in DNA, adenine (A) is complementary to thymine (T). For example, in RNA, adenine (A) is complementary to uracil (U). In certain embodiments, complementary nucleobase refers to a nucleobase of an antisense compound that is capable of base pairing with a nucleobase of its target nucleic acid. For example, if a nucleobase at a certain position of an antisense compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, then the position of hydrogen bonding

between the oligonucleotide and the target nucleic acid is considered to be complementary at that nucleobase pair.

"Nucleobase sequence" means the order of contiguous nucleobases independent of any sugar, linkage, and/or nucleobase modification.

"Nucleoside" means a nucleobase linked to a sugar.

"Nucleoside mimetic" includes those structures used to replace the sugar or the sugar and the base and not necessarily the linkage at one or more positions of an oligomeric compound such as for example nucleoside mimetics having morpholino, cyclohexenyl, cyclohexyl, tetrahydropyranyl, bicyclo or tricyclo sugar mimetics, *e.g.*, non furanose sugar units. Nucleotide mimetic includes those structures used to replace the nucleoside and the linkage at one or more positions of an oligomeric compound such as for example peptide nucleic acids or morpholinos (morpholinos linked by -N(H)-C(=O)-O- or other non-phosphodiester linkage). Sugar surrogate overlaps with the slightly broader term nucleoside mimetic but is intended to indicate replacement of the sugar unit (furanose ring) only. The tetrahydropyranyl rings provided herein are illustrative of an example of a sugar surrogate wherein the furanose sugar group has been replaced with a tetrahydropyranyl ring system. "Mimetic" refers to groups that are substituted for a sugar, a nucleobase, and/or internucleoside linkage. Generally, a mimetic is used in place of the sugar or sugar-internucleoside linkage combination, and the nucleobase is maintained for hybridization to a selected target.

"Nucleotide" means a nucleoside having a phosphate group covalently linked to the sugar portion of the nucleoside.

"Off-target effect" refers to an unwanted or deleterious biological effect associated with modulation of RNA or protein expression of a gene other than the intended target nucleic acid.

"Oligomeric compound" means a polymer of linked monomeric subunits which is capable of hybridizing to at least a region of a nucleic acid molecule.

"Oligonucleoside" means an oligonucleotide in which the internucleoside linkages do not contain a phosphorus atom.

"Oligonucleotide" means a polymer of linked nucleosides each of which can be modified or unmodified, independent one from another.

"Parenteral administration" means administration through injection (e.g., bolus injection) or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular

administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g., intrathecal or intracerebroventricular administration.

"Peptide" means a molecule formed by linking at least two amino acids by amide bonds. Without limitation, as used herein, "peptide" refers to polypeptides and proteins.

"Pharmaceutically acceptable carrier" means a medium or diluent that does not interfere with the structure of the oligonucleotide. Certain such carriers enable pharmaceutical compositions to be formulated as, for example, tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspension and lozenges for the oral ingestion by a subject.

"Pharmaceutically acceptable derivative" encompasses pharmaceutically acceptable salts, conjugates, prodrugs or isomers of the compounds described herein.

"Pharmaceutically acceptable salts" means physiologically and pharmaceutically acceptable salts of antisense compounds, i.e., salts that retain the desired biological activity of the parent oligonucleotide and do not impart undesired toxicological effects thereto.

"Pharmaceutical agent" means a substance that provides a therapeutic benefit when administered to an individual. For example, in certain embodiments, an antisense oligonucleotide targeted to HBV is a pharmaceutical agent.

"Pharmaceutical composition" means a mixture of substances suitable for administering to a subject. For example, a pharmaceutical composition may comprise an antisense oligonucleotide and a sterile aqueous solution. In certain embodiments, a pharmaceutical composition shows activity in free uptake assay in certain cell lines.

"Phosphorothioate linkage" means a linkage between nucleosides where the phosphodiester bond is modified by replacing one of the non-bridging oxygen atoms with a sulfur atom. A phosphorothioate linkage is a modified internucleoside linkage.

"Portion" means a defined number of contiguous (i.e., linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an antisense compound.

"Prevention" or "preventing" refers to delaying or forestalling the onset or development of a condition or disease for a period of time from hours to days, preferably weeks to months.

"Prodrug" means a therapeutic agent that is prepared in an inactive form that is converted to an active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions.

"Prophylactically effective amount" refers to an amount of a pharmaceutical agent that provides a prophylactic or preventative benefit to an animal.

"Recommended therapy" means a therapeutic regimen recommended by a medical professional for the treatment, amelioration, or prevention of a disease.

"Region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic.

"Ribonucleotide" means a nucleotide having a hydroxy at the 2' position of the sugar portion of the nucleotide. Ribonucleotides may be modified with any of a variety of substituents.

"Salts" mean a physiologically and pharmaceutically acceptable salts of antisense compounds, i.e., salts that retain the desired biological activity of the parent oligonucleotide and do not impart undesired toxicological effects thereto.

"Segments" are defined as smaller or sub-portions of regions within a target nucleic acid.

"Seroconversion" is defined as serum HBeAg absence plus serum HBeAb presence, if monitoring HBeAg as the determinant for seroconversion, or defined as serum HBsAg absence, if monitoring HBsAg as the determinant for seroconversion, as determined by currently available detection limits of commercial ELISA systems.

"Shortened" or "truncated" versions of antisense oligonucleotides taught herein have one, two or more nucleosides deleted.

"Side effects" means physiological responses attributable to a treatment other than desired effects. In certain embodiments, side effects include, without limitation, injection site reactions, liver function test abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, and myopathies. For example, increased aminotransferase levels in serum may indicate liver toxicity or liver function abnormality. For example, increased bilirubin may indicate liver toxicity or liver function abnormality.

"Significant," as used herein means measurable or observable, e.g, a significant result, such as, a significant improvement or significant reduction generally refers to a measurable or observable result, such as a measurable or observable improvement or reduction.

"Sites," as used herein, are defined as unique nucleobase positions within a target nucleic acid.

"Slows progression" means decrease in the development of the said disease.

"Specifically hybridizable" refers to an antisense compound having a sufficient degree of complementarity between an antisense oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays and therapeutic treatments. "Stringent hybridization conditions" or "stringent conditions" refer to conditions under which an oligomeric compound will hybridize to its target sequence, but to a minimal number of other sequences.

"Statistically Significant," as used herein means a measurable or observable parameter that is unlikely to occur by chance.

"Subcutaneous administration" means administration just below the skin.

"Subject" means a human or non-human animal selected for treatment or therapy.

"Target" refers to a protein, the modulation of which is desired.

"Target gene" refers to a gene encoding a target.

"Targeting" means the process of design and selection of an antisense compound that will specifically hybridize to a target nucleic acid and induce a desired effect.

"Target nucleic acid," "target RNA," "target RNA transcript" and "nucleic acid target" all mean a nucleic acid capable of being targeted by antisense compounds.

"Target region" means a portion of a target nucleic acid to which one or more antisense compounds is targeted.

"Target segment" means the sequence of nucleotides of a target nucleic acid to which an antisense compound is targeted. "5' target site" refers to the 5'-most nucleotide of a target segment. "3' target site" refers to the 3'-most nucleotide of a target segment.

"Therapeutically effective amount" means an amount of a pharmaceutical agent that provides a therapeutic benefit to an individual.

"Treatment" refers to administering a composition to effect an alteration or improvement of the disease or condition.

"Unmodified" nucleobases mean the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U).

"Unmodified nucleotide" means a nucleotide composed of naturally occuring nucleobases, sugar moieties, and internucleoside linkages. In certain embodiments, an unmodified nucleotide is an RNA nucleotide (i.e. β-D-ribonucleosides) or a DNA nucleotide (i.e. β-D-deoxyribonucleoside).

"Validated target segment" is defined as at least an 8-nucleobase portion (i.e. 8 consecutive nucleobases) of a target region to which an active oligomeric compound is targeted.

"Wing segment" means a plurality of nucleosides modified to impart to an oligonucleotide properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by *in vivo* nucleases.

Certain Embodiments

Certain embodiments provide methods, compounds, and compositions for inhibiting HBV mRNA expression.

Certain embodiments provide antisense compounds targeted to a HBV nucleic acid. In certain embodiments, the HBV nucleic acid is the sequences set forth in GENBANK Accession No. U95551.1 (incorporated herein as SEQ ID NO: 1).

In certain embodiments, the compounds provided herein are or comprise a modified oligonucleotide. In certain embodiments the compounds comprise a modified oligonucleotide and a conjugate as described herein. In certain embodiments, the modified oligonucleotide is a pharmaceutically acceptable derivative.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV. The HBV target can have a sequence recited in SEQ ID NO: 1 or a portion thereof or a variant thereof.

In certain embodiments, the compounds or modified oligonucleotides provided herein are 10 to 30 linked nucleosides in length and are targeted to HBV. In certain embodiments, the HBV target has the sequence recited in SEQ ID NO: 1. In certain embodiments, such compounds or oligonucleotides target one

of the following nucleotide regions of HBV: CCTGCTGGTGGCTCCAGTTC (SEQ ID NO: 1273); AGAGTCTAGACTCGTGGTGGACTTCTCTCA (SEQ ID NO: 1354); CATCCTGCTGCTATGCCTCATCTTCTT (SEQ ID NO: 1276); CAAGGTATGTTGCCCGT (SEQ ID NO: 1277); CCTATGGGAGTGGGCCTCAG (SEQ ID NO: 1279; TGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCG (SEQ ID NO: 1287); TATATGGATGATGTGGT (SEQ ID NO:1359); TGCCAAGTGTTTGCTGA (SEQ ID NO:1360); TGCCGATCCATACTGCGGAACTCCT (SEQ ID NO: 1361); CCGTGTGCACTTCGCTTCACCTCTGCACGT (SEQ ID NO:1352); GGAGGCTGTAGGCATAAATTGGT (SEQ ID NO:1353); CTTTTTCACCTCTGCCTA (SEQ ID NO:1362); TTCAAGCCTCCAAGCTGTGCCTTGG (SEQ ID NO:1363); AGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGG (SEQ ID NO: 1274); TGGATGTGTCTGCGGCGTTTTATCAT (SEQ ID NO: 1275); TGTATTCCCATCCCATC (SEQ ID NO: 1278); TGGCTCAGTTTACTAGTGC (SEQ ID NO: 1280); GGGCTTTCCCCCACTGT (SEQ ID NO: 1281); TCCTCTGCCGATCCATACTGCGGAACTCCT (SEQ ID NO: 1282); CGCACCTCTCTTTACGCGG (SEQ ID NO: 1283); GGAGTGTGGATTCGCAC (SEQ ID NO: 1284); or GAAGAAGAACTCCCTCGCCT (SEQ ID NO: 1285). In certain embodiments, such compounds or oligonucleotides have a gap segment of 9, 10, or more linked deoxynucleosides. In certain embodiments, such compounds or oligonucleotides have a gap segment of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 linked nucleosides. In certain embodiments, such gap segment is between two wing segments that independently have 1, 2, 3, 4, 5, 6, 7, or 8 linked modified nucleosides. In certain embodiments, one or more modified nucleosides in the wing segment have a modified sugar. In certain embodiments, the modified sugar is a bicyclic sugar. In certain embodiments, the modified nucleoside is an LNA nucleoside. In certain embodiments, the modified nucleoside is a 2'-substituted nucleoside. In certain embodiments, 2' substituted nucleosides include nucleosides with bicyclic sugar modifications. In certain embodiments, the modified nucleoside is a 2'-MOE nucleoside. In certain embodiments, the modified nucleoside is a constrained ethyl (cEt) nucleoside.

In certain embodiments, the compounds or compositions comprise modified oligonucleotides consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising a portion at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous nucleobases complementary to an equal length portion of any of the nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1. In certain embodiments, such oligonucleotides have a gap segment of 9, 10, or more linked deoxynucleosides. In certain embodiments, such gap segment is between two wing segments that independently have 1, 2, 3, 4, 5, 6, 7, or 8 linked modified nucleosides. In certain embodiments, one or more modified nucleosides in the

wing segment have a modified sugar. In certain embodiments, the modified sugar is a bicyclic sugar. In certain embodiments, the modified nucleoside is an LNA nucleoside. In certain embodiments, the modified nucleoside is a 2'-substituted nucleoside. In certain embodiments, 2' substituted nucleosides include nucleosides with bicyclic sugar modifications. In certain embodiments, the modified nucleoside is a 2'-MOE nucleoside. In certain embodiments, the modified nucleoside is a constrained ethyl (cEt) nucleoside. In certain embodiments, each modified nucleoside in each wing segment is independently a 2'-MOE nucleoside or a nucleoside with a bicyclic sugar modification such as a constrained ethyl (cEt) nucleoside or LNA nucleoside.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 5-310, 321-802, 804-1272, 1288-1350, 1364-1372, 1375, 1376, or 1379. In certain embodiments, such oligonucleotides have a gap segment of 9, 10, or more linked deoxynucleosides. In certain embodiments, such gap segment is between two wing segments that independently have 1-5, 1-4, 1-3, 2-5, 2-4 or 2-3 linked modified nucleosides. In certain embodiments, such gap segment is between two wing segments that independently have 1, 2, 3, 4, 5, 6, 7, or 8 linked modified nucleosides. In certain embodiments, one or more modified nucleosides in the wing segment have a modified sugar. In certain embodiments, the modified sugar is a bicyclic sugar. In certain embodiments, the modified nucleoside is an LNA nucleoside. In certain embodiments, the modified nucleoside is a 2'-substituted nucleoside. In certain embodiments, 2' substituted nucleosides include nucleosides with bicyclic sugar modifications. In certain embodiments, the modified nucleoside is a 2'-MOE nucleoside. In certain embodiments, the modified nucleoside is a constrained ethyl (cEt) nucleoside.

In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, or 4 sugar modified nucleosides. In certain embodiments, each sugar modified nucleoside is independently a 2'-MOE nucleoside or a nucleoside with a bicyclic sugar moiety such as a constrained ethyl (cEt) nucleoside or LNA nucleoside. In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 9 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, or 5 sugar modified nucleosides. In certain embodiments, each sugar modified nucleoside is independently a 2'-MOE nucleoside or a bicyclic nucleoside such as a constrained ethyl (cEt) nucleoside or LNA nucleoside.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11,

12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 5-310, 321-802, 804-1272, 1288-1350, 1364-1372, 1375, 1376, or 1379. In certain embodiments, such oligonucleotides have a gap segment of 10 or more linked deoxynucleosides. In certain embodiments, such gap segment is between two wing segments that independently have 1-5, 1-4, 1-3, 2-5, 2-4 or 2-3 linked modified nucleosides. In certain embodiments, such gap segment is between two wing segments that independently have 2, 3, 4, 5, 6, 7, or 8 linked modified nucleosides. In certain embodiments, one or more modified nucleosides in the wing segment have a modified sugar. In certain embodiments, the modified sugar is a bicyclic sugar. In certain embodiments, the modified nucleoside is an LNA nucleoside. In certain embodiments, the modified nucleoside is a 2'-substituted nucleoside. In certain embodiments, 2' substituted nucleosides include nucleosides with bicyclic sugar modifications. In certain embodiments, the modified nucleoside is a 2'-MOE nucleoside.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 5, 15, 16, 33, 39-95, 123-135, 163-175, 180-310, 321-406, 413-455, 461-802, or 804-1272. In certain embodiments, such oligonucleotides have a gap segment of 10 or more linked deoxynucleosides. In certain embodiments, such gap segment is between two wing segments that independently have 1-5, 1-4, 1-3, 2-5, 2-4 or 2-3 linked modified nucleosides. In certain embodiments, one or more modified nucleosides in the wing segment have a modified sugar. In certain embodiments, the modified sugar is a bicyclic sugar. In certain embodiments, the modified nucleoside is an LNA nucleoside. In certain embodiments, the modified nucleoside is a 2'-substituted nucleoside. In certain embodiments, 2' substituted nucleosides include nucleosides with bicyclic sugar modifications. In certain embodiments, the modified nucleoside is a 2'-MOE nucleoside.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 6-14, 17-32, 34-38, 96-122, 136-162, 176-179, 407-412, 456-462, 523-538. In certain embodiments, such oligonucleotides have a gap segment of 10 or more linked deoxynucleosides. In certain embodiments, such gap segment is between two wing segments that independently have 1-5, 1-4, 1-3, 2-5, 2-4 or 2-3 linked modified nucleosides. In certain embodiments, one or more modified nucleosides in the wing segment have a modified sugar. In certain embodiments, the modified nucleoside is a 2'-substituted nucleoside. In certain embodiments, the modified nucleoside is a 2'-MOE nucleoside. In certain embodiments, the modified oligonucleotide is 14 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-3 or 2 sugar modified nucleosides.

In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 2-4 or 3 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 9 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, or 5 sugar modified nucleosides, such as 2'-MOE nucleosides.

In certain embodiments, the modified oligonucleotide is 17 nucleosides in length and has a gap segment of 9 or 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 2-4 or 3-4 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, 5, or 6 sugar modified nucleosides, such as 2'-MOE nucleosides.

In certain embodiments, the modified oligonucleotide is 18 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 3-5, or 4 sugar modified nucleosides.

In certain embodiments, the modified oligonucleotide is 20 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, or 5 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, 5, 6, 7, or 8 sugar modified nucleosides, such as 2'-MOE nucleosides.

In certain embodiments, the compounds or compositions comprise a salt of the modified oligonucleotide.

In certain embodiments, the compounds or compositions further comprise a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 70%, 75%, 80%, 85%, 90%, 95% or 100% complementary to SEQ ID NO: 1, as measured over the entirety of the modified oligonucleotide.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 70%, 75%, 80%, 85%, 90%, 95% or 100% complementary to any one of SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, as measured over the entirety of the modified oligonucleotide.

In certain embodiments, the compound or modified oligonucleotide is single-stranded.

In certain embodiments, the modified oligonucleotide consists of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 18 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 14 linked nucleosides.

In certain embodiments, at least one internucleoside linkage of the modified oligonucleotide is a modified internucleoside linkage. In certain embodiments, each internucleoside linkage is a phosphorothioate internucleoside linkage.

In certain embodiments, at least one nucleoside of the modified oligonucleotide comprises a modified sugar. In certain embodiments, at least one modified sugar comprises a 2'-O-methoxyethyl group (2'-$O(CH_2)_2$-OCH_3). In certain embodiments, the modified sugar comprises a 2'-O-CH_3 group.

In certain embodiments, at least one modified sugar is a bicyclic sugar. In certain embodiments, at least one modified sugar the bicyclic sugar comprises a 4'- $(CH_2)_n$-O-2' bridge, wherein n is 1 or 2. In certain embodiments, the bicyclic sugar comprises a 4'- CH_2-O-2' bridge. In certain embodiments, the bicyclic sugar comprises a 4'-$CH(CH_3)$-O-2' bridge.

In certain embodiments, at least one nucleoside of said modified oligonucleotide comprises a modified nucleobase. In certain embodiments, the modified nucleobase is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of a single-stranded modified oligonucleotide.

In certain embodiments, the modified oligonucleotide comprises: a) a gap segment consisting of linked deoxynucleosides; b) a 5' wing segment consisting of linked nucleosides; and c) a 3' wing segment consisting of linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment and each nucleoside of each wing segment comprises a modified sugar.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, the gap segment consisting of 10 linked deoxynucleosides, the 5' wing segment consisting of three linked nucleosides, the 3' wing segment consisting of three linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar and/or a constrained ethyl (cEt) sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine. In some aspects, each of the three linked nucleosides of the 5' wing segment is a 2'-O-methoxyethyl sugar and each of the three linked nucleosides of the 3' wing segment is a constrained ethyl (cEt) sugar. In other aspects, the three linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a

constrained ethyl (cEt) sugar in the 5' to 3' direction, and the three linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction. In other aspects, the three linked nucleosides of the 5' wing segment are a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction, and the three linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, the gap segment consisting of 10 linked deoxynucleosides, the 5' wing segment consisting of two linked nucleosides, the 3' wing segment consisting of four linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar and a constrained ethyl (cEt) sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine. In some aspects, the two linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar and a constrained ethyl (cEt) sugar in the 5' to 3' direction, and the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, the gap segment consisting of 9 linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of two linked nucleosides; the five linked nucleosides of the 5' wing segment are a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside, a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the two linked nucleosides of the 3' wing segment are a 2'-O-methoxyethyl sugar and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, the gap segment consisting of 8 linked deoxynucleosides, the 5' wing segment consisting of three linked nucleosides, the 3' wing segment consisting of five linked nucleosides; the three linked nucleosides of the 5' wing segment are a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; each of the five linked nucleosides of the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, the gap segment consisting of 8 linked deoxynucleosides, the 5' wing segment consisting of three linked nucleosides, the 3' wing segment consisting of five linked nucleosides; each of the three linked nucleosides of the 5' wing segment is a constrained ethyl (cEt) sugar; each of the five linked nucleosides of the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of 9 linked deoxynucleosides, the 5' wing segment consisting of four linked nucleosides, the 3' wing segment consisting of four linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar and a constrained ethyl (cEt) sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine. In some aspects, the four linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction, and the four linked nucleosides of the 3' wing segment are a 2'-O-methoxyethyl sugar, constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of 9 linked deoxynucleosides, the 5' wing segment consisting of four linked nucleosides, the 3' wing segment consisting of four linked nucleosides; the four linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, and a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of 9 linked deoxynucleosides, the 5' wing segment consisting of four linked nucleosides, the 3' wing segment consisting of four linked nucleosides; the four linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; each of the four linked nucleosides of the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of 8 linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of four linked nucleosides; the five linked nucleosides of the 5' wing segment are a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside, a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; each of the four linked nucleosides of the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of 8 linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of four linked nucleosides; the five linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; each of the four linked nucleosides of

the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of 7 linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of five linked nucleosides; the five linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the five linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, and a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of 7 linked deoxynucleosides, the 5' wing segment consisting of six linked nucleosides, the 3' wing segment consisting of four linked nucleosides; the six linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; each of the four linked nucleosides of the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of 7 linked deoxynucleosides, the 5' wing segment consisting of six linked nucleosides, the 3' wing segment consisting of four linked nucleosides; the six linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; each of the four linked nucleosides of the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of 10 linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of five linked nucleosides, each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of 10 linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of five linked nucleosides, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine, wherein the five linked nucleosides of the 5' wing are a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside, a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside,

and a constrained ethyl (cEt) sugar in the 5' to 3' direction, and each of the five linked nucleosides of the 3' wing are a 2'-O-methoxyethyl sugar.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of three linked nucleosides; and c) a 3' wing segment consisting of three linked nucleosides. In some aspects, the gap segment is positioned between the 5' wing segment and the 3' wing segment; each of the three linked nucleosides of the 5' wing segment is a 2'-O-methoxyethyl sugar and each of the three linked nucleosides of the 3' wing segment is a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine residue is a 5-methylcytosine. In other aspects, the gap segment is positioned between the 5' wing segment and the 3' wing segment; the three linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the three linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine residue is a 5-methylcytosine. In other aspects, the three linked nucleosides of the 5' wing segment are a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction, and the three linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of two linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides. In some aspects, the gap segment is positioned between the 5' wing segment and the 3' wing segment; the two linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, 2'-O-methoxyethyl sugar, constrained ethyl (cEt) sugar, and 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) gap segment consisting of 9 linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of two linked nucleosides, wherein the five linked nucleosides of the 5' wing segment are a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside, a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the two linked nucleosides of the 3' wing segment are a 2'-O-methoxyethyl sugar and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of 8 linked deoxynucleosides; b) a 5' wing segment consisting of three linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides, wherein the three linked nucleosides of the 5' wing segment are a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; each of the five linked nucleosides of the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of 8 linked deoxynucleosides; b) a 5' wing segment consisting of three linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides, wherein each of the three linked nucleosides of the 5' wing segment is a constrained ethyl (cEt) sugar; each of the five linked nucleosides of the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of 9 linked deoxynucleosides; b) a 5' wing segment consisting of four linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar and a constrained ethyl (cEt) sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine. In some aspects, the four linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction, and the four linked nucleosides of the 3' wing segment are a 2'-O-methoxyethyl sugar, constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of 9 linked deoxynucleosides; b) a 5' wing segment consisting of four linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides, wherein the four linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, and a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of 9 linked deoxynucleosides; b) a 5' wing segment consisting of four linked nucleosides; and c) the 3' wing segment consisting of four linked nucleosides, wherein the four linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a constrained ethyl (cEt)

sugar in the 5' to 3' direction; each of the four linked nucleosides of the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of 8 linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides, wherein the five linked nucleosides of the 5' wing segment are a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside, a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; each of the four linked nucleosides of the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of 8 linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides, wherein the five linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; each of the four linked nucleosides of the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of 7 linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides, wherein the five linked nucleosides of the 5' wing segment are a 2'-O-

methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the five linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, and a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of 7 linked deoxynucleosides; b) a 5' wing segment consisting of six linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides, wherein the six linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; each of the four linked nucleosides of the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of 7 linked deoxynucleosides; b) a 5' wing segment consisting of six linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides, wherein the six linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; each of the four linked nucleosides of the 3' wing segment is a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of 10 linked

deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides, each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of 10 linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine, wherein the five linked nucleosides of the 5' wing are a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside, a constrained ethyl (cEt) sugar, a 2'-deoxynucleoside, and a constrained ethyl (cEt) sugar in the 5' to 3' direction, and each of the five linked nucleosides of the 3' wing are a 2'-O-methoxyethyl sugar.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of ten linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of five linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of ten linked deoxynucleosides, the 5' wing segment consisting of two linked nucleosides, the 3' wing segment consisting of eight linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of ten linked deoxynucleosides, the 5' wing segment consisting of eight linked nucleosides, the 3' wing segment consisting of two linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of ten linked deoxynucleosides, the 5' wing segment consisting of three linked nucleosides, the 3' wing segment consisting of seven linked nucleosides, each nucleoside of each wing

segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of ten linked deoxynucleosides, the 5' wing segment consisting of seven linked nucleosides, the 3' wing segment consisting of three linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of ten linked deoxynucleosides, the 5' wing segment consisting of four linked nucleosides, the 3' wing segment consisting of six linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of ten linked deoxynucleosides, the 5' wing segment consisting of six linked nucleosides, the 3' wing segment consisting of four linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 18 linked nucleosides, the gap segment consisting of ten linked deoxynucleosides, the 5' wing segment consisting of four linked nucleosides, the 3' wing segment consisting of four linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of ten linked deoxynucleosides, the 5' wing segment consisting of three linked nucleosides, the 3' wing segment consisting of four linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of nine linked deoxynucleosides, the 5' wing segment consisting of two linked nucleosides, the 3' wing segment consisting of six linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of nine linked deoxynucleosides, the 5' wing segment consisting of six linked nucleosides, the 3' wing segment consisting of two linked nucleosides, each nucleoside of each wing segment

comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of nine linked deoxynucleosides, the 5' wing segment consisting of four linked nucleosides, the 3' wing segment consisting of four linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of nine linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of three linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides, the gap segment consisting of nine linked deoxynucleosides, the 5' wing segment consisting of three linked nucleosides, the 3' wing segment consisting of five linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, the gap segment consisting of ten linked deoxynucleosides, the 5' wing segment consisting of three linked nucleosides, the 3' wing segment consisting of three linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, the gap segment consisting of nine linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of two linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, the gap segment consisting of nine linked deoxynucleosides, the 5' wing segment consisting of two linked nucleosides, the 3' wing segment consisting of five linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, the gap segment consisting of nine linked deoxynucleosides, the 5' wing segment consisting of four linked nucleosides, the 3' wing segment consisting of three linked nucleosides, each nucleoside of each wing

segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, the gap segment consisting of nine linked deoxynucleosides, the 5' wing segment consisting of three linked nucleosides, the 3' wing segment consisting of four linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 14 linked nucleosides, the gap segment consisting of ten linked deoxynucleosides, the 5' wing segment consisting of two linked nucleosides, the 3' wing segment consisting of two linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of two linked nucleosides; and c) a 3' wing segment consisting of eight linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising a portion of at least 8

contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of eight linked nucleosides; and c) a 3' wing segment consisting of two linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of three linked nucleosides; and c) a 3' wing segment consisting of seven linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of seven linked nucleosides; and c) a 3' wing segment consisting of three linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked

deoxynucleosides; b) a 5' wing segment consisting of four linked nucleosides; and c) a 3' wing segment consisting of six linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of six linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 18 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of four linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of three linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of nine linked deoxynucleosides; b) a 5' wing segment consisting of two linked nucleosides; and c) a 3' wing segment consisting of six linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of nine linked deoxynucleosides; b) a 5' wing segment consisting of six linked nucleosides; and c) a 3' wing segment consisting of two linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of nine linked deoxynucleosides; b) a 5' wing segment consisting of four linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353,

1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of nine linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of three linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 17 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of nine linked deoxynucleosides; b) a 5' wing segment consisting of three linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of three linked nucleosides; and c) a 3' wing segment consisting of three linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of nine linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of two linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3'

wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of nine linked deoxynucleosides; b) a 5' wing segment consisting of two linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of nine linked deoxynucleosides; b) a 5' wing segment consisting of four linked nucleosides; and c) a 3' wing segment consisting of three linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of nine linked deoxynucleosides; b) a 5' wing segment consisting of three linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 14 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of any of nucleobases set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, wherein the nucleobase sequence is complementary to SEQ ID NO: 1 and wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of two linked nucleosides; and c) a 3' wing segment consisting of two linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the provided methods, compounds, and compositions inhibit HBV mRNA expression and/or DNA levels and or protein levels and/or antigen levels.

Another embodiment provides a method for treating a HBV-related diseases, disorders, and conditions in a mammal, the method comprising administering a therapeutically effective amount of any pharmaceutical composition as described above to a mammal in need thereof, so as to treat the HBV-related diseases, disorders, and condition. In related embodiments, the mammal is a human and the HBV-related disease, disorder, and condition is a hepatitis B virus infection from a human hepatitis B virus. More particularly, the human hepatitis B virus may be any of the human geographical genotypes: A (Northwest Europe, North America, Central America); B (Indonesia, China, Vietnam); C (East Asia, Korea, China, Japan, Polynesia, Vietnam); D (Mediterranean area, Middle East, India); E (Africa); F (Native Americans, Polynesia); G (United States, France); or H (Central America).

In certain embodiments, an antisense compound or oligonucleotide targeted to a HBV nucleic acid is complementary within the following nucleotide regions of SEQ ID NO: 1: 1-20, 10-29, 10-56, 13-38, 13-35, 19-38, 25-47, 25-50, 25-56, 43-68, 43-63, 55-74, 58-73, 58-74, 58-77, 58-79, 58-80, 58-84, 59-74, 59-75, 59-80, 60-75, 60-76, 60-79, 61-76, 61-77, 61-80, 62-77, 63-84, 68-114, 101-123, 98-123, 113-138, 116-138, 131-150, 137-162, 152-186, 158-177, 167-186, 191-215, 196-224, 196-215, 196-218, 199-228, 199-218, 199-224, 200-224, 205-224, 206-228, 218-237, 224-243, 233-264, 242-263, 243-262, 244-263, 245-274; 245-260, 245-264, 246-266, 247-266, 247-269, 247-270, 245-267, 251-267, 245-266, 250-269, 251-266, 251-268, 251-269, 245-269, 245-266, 245-261, 250-265, 250-266, 250-267, 250-268, 250-269, 251-270, 252-267, 253-268, 253-269, 253-272, 253-274, 254-269, 254-270, 254-274, 255-270, 255-271, 255-274, 255-401, 255-400, 255-274, 255-273, 255-272, 255-271, 256-271, 256-275, 255-276, 256-272, 256-276, 253-275, 256-279, 257-276, 258-273, 259-274, 260-279, 262-281, 262-321, 262-315, 262-312, 265-312, 266-288, 266-291, 266-285, 281-321, 281-303, 290-321, 290-312, 292-311, 290-312, 293-312, 293-315, 293-321, 296-321, 302-321, 324-343, 339-361, 339-367, 348-367, 342-367, 358-392, 358-378, 360-392, 360-383, 360-388, 360-385, 362-381,

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In certain embodiments, an antisense compound or oligonucleotide targeted to a HBV nucleic acid target the following nucleotide regions of SEQ ID NO: 1: 1-20, 10-29, 10-56, 13-38, 13-35, 19-38, 25-47, 25-50, 25-56, 43-68, 43-63, 55-74, 58-73, 58-74, 58-77, 58-79, 58-80, 58-84, 59-74, 59-75, 59-80, 60-75, 60-76, 60-79, 61-76, 61-77, 61-80, 62-77, 63-84, 68-114, 101-123, 98-123, 113-138, 116-138, 131-150, 137-162, 152-186, 158-177, 167-186, 191-215, 196-224, 196-215, 196-218, 199-228, 199-218, 199-224, 200-224, 205-224, 206-228, 218-237, 224-243, 233-264, 242-263, 243-262, 244-263, 245-274; 245-260, 245-264, 246-266, 247-266, 247-269, 247-270, 245-267, 251-267, 245-266, 250-269, 251-266, 251-268, 251-269, 245-269, 245-266, 245-261, 250-265, 250-266, 250-267, 250-268, 250-269, 251-270, 252-267, 253-268, 253-269, 253-272, 253-274, 254-269, 254-270, 254-274, 255-270, 255-271, 255-274, 255-401, 255-400, 255-274, 255-273, 255-272, 255-271, 256-271, 256-275, 255-276, 256-272, 256-276, 253-275, 256-279, 257-276, 258-273, 259-274, 260-279, 262-281, 262-321, 262-315, 262-312, 265-312, 266-288, 266-291, 266-285, 281-321, 281-303, 290-321, 290-312, 292-311, 290-312, 293-312, 293-315, 293-321, 296-321, 302-321, 324-343, 339-361, 339-367, 348-367, 342-367, 358-392, 358-378, 360-392, 360-383, 360-388, 360-385, 362-381, 366-388, 369-388, 366-385, 366-392, 370-389, 370-392, 380-399, 382-401, 384-433, 384-400, 384-401, 385-401, 405-424, 409-428,

405-428, 411-426, 411-427, 411-430, 411-431, 411-437, 412-431, 411-426, 411-427, 412-428, 412-431, 412-427, 413-433, 413-432, 413-428, 413-429, 413-432, 413-433, 414-427, 415-427, 414-429, 414-430, 414-433, 415-428, 415-429, 415-430, 415-431, 415-434, 416-431, 416-432, 416-429, 416-435, 417-432, 417-433, 417-436, 418-433, 418-435, 418-434, 418-437, 419-435, 419-434, 420-435, 419-432, 419-434, 421-436, 422-437, 422-441, 423-436, 425-465, 454-473, 454-472, 457-476, 457-472, 457-473, 454-476, 455-472, 457-485, 458-485, 458-483, 458-477, 458-473, 459-485, 460-485, 463-498, 463-485, 466-485, 463-482, 457-491, 458-491, 459-491, 460-491, 463-491, 466-491, 472-491, 472-493, 473-492, 475-491, 459-494, 460-494, 463-494, 466-494, 467-498, 472-494, 475-494, 457-473, 457-472, 458-494, 454-494, 457-494, 457-473, 485-513, 470-493, 476-519, 485-519, 500-519, 512-534, 512-550, 524-546, 536-559, 548-567, 548-570, 550-570, 548-594, 554-573, 548-576, 560-594, 584-606, 611-645, 617-363, 623-642, 617-645, 639-754, 639-658, 639-654, 641-656, 642-657, 643-658, 642-754, 653-672, 662-685, 665-685,665-689, 668-687, 670-754, 670-706, 670-685, 670-686, 670-689, 671-690, 671-691, 671-686, 671-687, 672-693, 672-697, 672-707, 672-687, 672-688, 673-688, 674-693, 678-693, 679-694, 679-707, 679-698, 679-701, 679-702, 679-707, 680-695, 680-699, 679-699, 681-706, 681-696, 682-697, 682-706, 682-707, 682-702, 682-701, 683-698, 684-699, 685-700, 686-701, 687-754, 688-704, 689-709, 689-710, 690-705, 679-705, 679-710, 679-706, 690-710, 691-710, 690-754, 690-706, 684-703, 687-705, 687-702, 687-703, 687-706, 688-703, 688-704, 688-705, 689-704, 689-705, 689-708, 690-705, 690-706, 690-709, 691-706, 692-711, 693-716, 693-712, 695-715, 697-716, 697-716, 690-716, 724-746, 724-752, 724-754, 724-758, 733-752, 738-754, 738-753, 739-758, 739-754, 739-775, 739-754, 740-754, 742-785, 742-773, 757-776, 757-785, 790-815, 793-812, 811-833, 811-844, 814-833, 811-906, 820-839, 822-844, 822-867, 823-842, 845-864, 845-867, 854-906, 845-909, 845-906, 854-876, 863-882, 863-885, 878-900, 887-906, 899-918, 899-933, 899-958, 905-927, 905-933, 914-933, 936-958, 936-955, 945-964, 951-970, 951-985, 951-1044, 951-1024, 951-1056, 951-997, 960-985, 963-1044, 963-1024, 963-997, 972-1015, 1025-1044, 1031-1056, 1037-1056, 1046-1083, 1049-1068, 1070-1089, 1070-1095, 1082-1101, 1081-1134, 1081-1143, 1082-1101, 1088-1107, 1088-1134, 1094-1119, 1097-1119, 1112-1134, 1118-1143, 1118-1146, 1088-1146, 1121-1140, 1127-1146, 1127-1193, 1150-1193, 1156-1187, 1165-1187, 1170-1192, 1171-1191, 1172-1191, 1176-1192, 1176-1285, 1177-1192, 1176-1191, 1203-1297, 1206-1228, 1206-1255, 1209-1228, 1215-1255, 1245-1265, 1251-1280, 1262-1285, 1251-1285, 1259-1296, 1259-1290, 1259-1287, 1261-1296, 1261-1285, 1261-1276, 1261-1277, 1261-1280, 1262-1296, 1262-1277, 1262-1278, 1262-1281, 1263-1278, 1263-1279, 1263-1282, 1264-1279, 1264-1280, 1264-1283, 1264-1297, 1265-1280, 1265-1281, 1265-1284, 1266-1281, 1266-1282, 1266-1285, 1267-1282, 1267-1283, 1268-1283, 1268-1284, 1268-1296, 1269-1284, 1269-1285, 1269-1288, 1270-1285, 1271-1290, 1271-1296, 1277-1296, 1261-1290, 1262-1290, 1268-1290, 1263-1305, 1259-1305, 1259-1305, 1266-1305, 1259-1302, 1275-1294, 1281-1306, 1281-1324, 1281-1336, 1282-1301, 1286-1306, 1290-1324, 1293-1318, 1290-1324, 1293-1315, 1296-1315, 1311-1336, 1311-1333, 1326-1345, 1353-1381, 1359-1378, 1395-1414, 1498-1532, 1498-1523, 1498-1535, 1510-1529, 1515-1535, 1515-1563, 1515-1596, 1515-1605, 1515-1602, 1515-1540, 1515-1535, 1518-1605, 1518-1602, 1518-1537, 1521-1563, 1521-

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In certain embodiments, an antisense compound or oligonucleotide targeted to a HBV nucleic acid is complementary within the HBV pre-S1 second portion gene region corresponding to nucleotide region 1-1932 of SEQ ID NO: 1. In certain embodiments, an antisense compound or oligonucleotide targeted to a HBV nucleic acid is complementary within the HBV pre-S1 first portion gene region corresponding to nucleotide region 2831-3182 of SEQ ID NO: 1.

In certain embodiments, an antisense compound or oligonucleotide targeted to a HBV nucleic acid target the HBV pre-S1 second portion gene region corresponding to nucleotide region 1-1932 of SEQ ID NO: 1. In certain embodiments, an antisense compound or oligonucleotide targeted to a HBV nucleic acid target the HBV pre-S1 first portion gene region corresponding to nucleotide region 2831-3182 of SEQ ID NO: 1.

In certain embodiments, antisense compounds or oligonucleotides target a region of a HBV nucleic acid. In certain embodiments, such compounds or oligonucleotides targeted to a region of a HBV nucleic acid have a contiguous nucleobase portion that is complementary to an equal length nucleobase portion of the region. For example, the portion can be at least an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobases portion complementary to an equal length portion of a region recited herein. In certain embodiments, such compounds or oligonucleotide target the following nucleotide regions of SEQ ID NO: 1:

1-20, 10-29, 10-56, 13-38, 13-35, 19-38, 25-47, 25-50, 25-56, 43-68, 43-63, 55-74, 58-73, 58-74, 58-77, 58-79, 58-80, 58-84, 59-74, 59-75, 59-80, 60-75, 60-76, 60-79, 61-76, 61-77, 61-80, 62-77, 63-84, 68-114, 101-123, 98-123, 113-138, 116-138, 131-150, 137-162, 152-186, 158-177, 167-186, 191-215, 196-224, 196-215, 196-218, 199-228, 199-218, 199-224, 200-224, 205-224, 206-228, 218-237, 224-243, 233-264, 242-263, 243-262, 244-263, 245-274; 245-260, 245-264, 246-266, 247-266, 247-269, 247-270, 245-267, 251-267, 245-266, 250-269, 251-266, 251-268, 251-269, 245-269, 245-266, 245-261, 250-265, 250-266, 250-267, 250-268, 250-269, 251-270, 252-267, 253-268, 253-269, 253-272, 253-274, 254-269, 254-270, 254-274, 255-270, 255-271, 255-274, 255-401, 255-400, 255-274, 255-273, 255-272, 255-271, 256-271, 256-275, 255-276, 256-272, 256-276, 253-275, 256-279, 257-276, 258-273, 259-274, 260-279, 262-281, 262-321, 262-315, 262-312, 265-312, 266-288, 266-291, 266-285, 281-321, 281-303, 290-321, 290-312, 292-311, 290-312, 293-312, 293-315, 293-321, 296-321, 302-321, 324-343, 339-361, 339-367, 348-367, 342-367, 358-392, 358-378, 360-392, 360-383, 360-388, 360-385, 362-381, 366-388, 369-388, 366-385, 366-392, 370-389, 370-392, 380-399, 382-401, 384-433, 384-400, 384-401, 385-401, 405-424, 409-428, 405-428, 411-426, 411-427, 411-430, 411-431, 411-437, 412-431, 411-426, 411-427, 412-428, 412-431, 412-427, 413-433, 413-432, 413-428, 413-429, 413-432, 413-433, 414-427, 415-427, 414-429, 414-430, 414-433, 415-428, 415-429, 415-430, 415-431, 415-434, 416-431, 416-432, 416-429, 416-435, 417-432, 417-433, 417-436, 418-433, 418-435, 418-434, 418-437, 419-435, 419-434, 420-435, 419-432, 419-434, 421-436, 422-437, 422-441, 423-436, 425-465, 454-473, 454-472, 457-476, 457-472, 457-473, 454-476, 455-472, 457-485, 458-485, 458-483, 458-477, 458-473, 459-485, 460-485, 463-498, 463-485, 466-485, 463-482, 457-491, 458-491, 459-491, 460-491, 463-491, 466-491, 472-491, 472-493, 473-492, 475-491, 459-494, 460-494, 463-494, 466-494, 467-498, 472-494, 475-494, 457-473, 457-472, 458-494, 454-494, 457-494, 457-473, 485-513, 470-493, 476-519, 485-519, 500-519, 512-534, 512-550, 524-546, 536-559, 548-567, 548-570, 550-570, 548-594, 554-573, 548-576, 560-594, 584-606, 611-645, 617-363, 623-642, 617-645, 639-754, 639-658, 639-654, 641-656, 642-657, 643-658, 642-754, 653-672, 662-685, 665-685,665-689, 668-687, 670-754, 670-706, 670-685, 670-686, 670-689, 671-690, 671-691, 671-686, 671-687, 672-693, 672-697, 672-707, 672-687, 672-688, 673-688, 674-693, 678-693, 679-694, 679-707, 679-698, 679-701, 679-702, 679-707, 680-695, 680-699, 679-699, 681-706, 681-696, 682-697, 682-706, 682-707, 682-702, 682-701, 683-698, 684-699, 685-700, 686-701, 687-754, 688-704, 689-709, 689-710, 690-705, 679-705, 679-710, 679-706, 690-710, 691-710, 690-754, 690-706, 684-703, 687-705, 687-702, 687-703, 687-706, 688-703, 688-704, 688-705, 689-704, 689-705, 689-708, 690-705, 690-706, 690-709, 691-706, 692-711, 693-716, 693-712, 695-715, 697-716, 697-716, 690-716, 724-746, 724-752, 724-754, 724-758, 733-752, 738-754, 738-753, 739-758, 739-754, 739-775, 739-754, 740-754, 742-785, 742-773, 757-776, 757-785, 790-815, 793-812, 811-833, 811-844, 814-833, 811-906, 820-839, 822-844, 822-867, 823-842, 845-864, 845-867, 854-906, 845-909, 845-906, 854-876, 863-882, 863-885, 878-900, 887-906, 899-918, 899-933, 899-958, 905-927, 905-933, 914-933, 936-958, 936-955, 945-964, 951-970, 951-985, 951-1044, 951-1024, 951-1056, 951-997, 960-985, 963-1044, 963-1024, 963-997, 972-1015, 1025-1044, 1031-1056, 1037-1056, 1046-1083, 1049-1068, 1070-

1089, 1070-1095, 1082-1101, 1081-1134, 1081-1143, 1082-1101, 1088-1107, 1088-1134, 1094-1119, 1097-1119, 1112-1134, 1118-1143, 1118-1146, 1088-1146, 1121-1140, 1127-1146, 1127-1193, 1150-1193, 1156-1187, 1165-1187, 1170-1192, 1171-1191, 1172-1191, 1176-1192, 1176-1285, 1177-1192, 1176-1191, 1203-1297, 1206-1228, 1206-1255, 1209-1228, 1215-1255, 1245-1265, 1251-1280, 1262-1285, 1251-1285, 1259-1296, 1259-1290, 1259-1287, 1261-1296, 1261-1285, 1261-1276, 1261-1277, 1261-1280, 1262-1296, 1262-1277, 1262-1278, 1262-1281, 1263-1278, 1263-1279, 1263-1282, 1264-1279, 1264-1280, 1264-1283, 1264-1297, 1265-1280, 1265-1281, 1265-1284, 1266-1281, 1266-1282, 1266-1285, 1267-1282, 1267-1283, 1268-1283, 1268-1284, 1268-1296, 1269-1284, 1269-1285, 1269-1288, 1270-1285, 1271-1290, 1271-1296, 1277-1296, 1261-1290, 1262-1290, 1268-1290, 1263-1305, 1259-1305, 1259-1305, 1266-1305, 1259-1302, 1275-1294, 1281-1306, 1281-1324, 1281-1336, 1282-1301, 1286-1306, 1290-1324, 1293-1318, 1290-1324, 1293-1315, 1296-1315, 1311-1336, 1311-1333, 1326-1345, 1353-1381, 1359-1378, 1395-1414, 1498-1532, 1498-1523, 1498-1535, 1510-1529, 1515-1535, 1515-1563, 1515-1596, 1515-1605, 1515-1602, 1515-1540, 1515-1535, 1518-1605, 1518-1602, 1518-1537, 1521-1563, 1521-1540, 1550-1655, 1550-1563, 1550-1569, 1553-1578, 1553-1599, 1553-1590, 1565-1584, 1571-1595, 1577-1605, 1577-1606, 1577-1592, 1577-1593, 1577-1596, 1578-1593, 1578-1594, 1578-1597, 1578-1598, 1571-1598, 1579-1594, 1579-1594, 1579-1598, 1580-1595, 1580-1596, 1580-1599, 1580-1605, 1580-1602, 1581-1596, 1581-1597, 1581-1600, 1582-1597, 1582-1598, 1582-1601, 1582-1602, 1553-1655, 1583-1598, 1583-1599, 1583-1602, 1584-1599, 1584-1600, 1584-1603, 1585-1600, 1585-1601, 1585-1604, 1586-1601, 1586-1602, 1586-1605, 1587-1602, 1587-1603, 1587-1606, 1588-1603, 1588-1604, 1589-1604, 1589-1605, 1590-1605, 1590-1606, 1591-1606, 1586-1652, 1642-1664, 1651-1720, 1651-1673, 1655-1679, 1695-1720, 1716-1738, 1743-1763, 1743-1768, 1764-1783, 1773-1792, 1777-1796, 1777-1800, 1778-1800, 1778-1793, 1778-1794, 1778-1797, 1779-1798, 1779-1797, 1779-1796, 1779-1795, 1779-1794, 1780-1799, 1780-1796, 1780-1795, 1781-1797, 1781-1796, 1781-1796, 1781-1800, 1781-1797, 1782-1799, 1782-1797, 1782-1798, 1783-1798, 1783-1799, 1784-1800, 1784-1799, 1779-1799, 1778-1889, 1778-1794, 1779-1795, 1780-1799, 1785-1800, 1794-1813, 1806-1837, 1806-1828, 1806-1825, 1809-1828, 1812-1843, 1812-1837, 1812-1831, 1815-1843, 1815-1844, 1815-1840, 1815-1834, 1818-1837, 1821-1840, 1821-1844, 1821-1837, 1822-1843, 1822-1839, 1822-1837, 1823-1843, 1823-1838, 1824-1839, 1827-1846, 1861-1884, 1861-1880, 1865-1885, 1866-1881, 1867-1882, 1867-1886, 1868-1883, 1869-1885, 1869-1884, 1870-1885, 1871-1886, 1872-1887, 1874-1889, 1876-1895, 1888-1914, 1888-1908, 1891-1910, 1891-1914, 1895-1938, 1895-1935, 1913-1935, 1898-1920, 1907-1929, 1913-1935, 1918-1934, 1919-1938, 1919-1934, 1921-1934, 1928-1956, 1957-1976, 2035-2057, 2083-2141, 2230-2261, 2368-2393, 2381-2397, 2368-2394, 2379-2394, 2381-2396, 2368-2397, 2368-2396, 2420-2439, 2458-2476, 2459-2478, 2819-2838, 2818-2838, 2873-2892, and 3161-3182..

In certain embodiments, antisense compounds or oligonucleotides target a region of a HBV nucleic acid. In certain embodiments, such compounds or oligonucleotides targeted to a region of a HBV nucleic acid have a contiguous nucleobase portion that is complementary to an equal length nucleobase portion of the

region. For example, the portion can be at least an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobases portion complementary to an equal length portion of a region recited herein. In certain embodiments, such compounds or oligonucleotide target the following nucleotide regions of SEQ ID NO: 1: 58-73, 58-74, 58-77, 59-74, 60-75, 61-76, 62-77, 245-274; 245-260, 250-265, 251-266, 252-267, 253-268, 254-269, 255-270, 256-271, 256-272, 258-273, 259-274, 380-399, 382-401, 411-437, 411-427, 411-426, 412-427, 413-428, 413-432, 414-429, 415-430, 416-431, 417-432, 418-433, 419-434, 420-435, 421-436, 422-437, 457-472, 458-473, 639-754, 639-658, 639-654, 641-656, 642-657, 643-658, 670-754, 670-706, 670-685, 671-686, 672-687, 673-688, 678-693, 679-694, 680-695, 681-706, 681-696, 682-697, 683-698, 684-699, 685-700, 686-701, 687-702, 688-703, 689-704, 690-705, 691-706, 738-754, 738-753, 739-754, 1176-1285, 1176-1191, 1177-1192, 1261-1285, 1261-1276, 1262-1277, 1263-1278, 1264-1279, 1265-1280, 1266-1281, 1267-1282, 1268-1283, 1269-1284, 1270-1285, 1577-1606, 1577-1592, 1578-1593, 1579-1594, 1580-1595, 1581-1596, 1582-1597, 1583-1598, 1584-1599, 1585-1600, 1586-1601, 1587-1602, 1588-1603, 1589-1604, 1590-1605, 1591-1606, 1778-1889, 1778-1800, 1778-1793, 1779-1794, 1780-1799, 1780-1796, 1780-1795, 1781-1796, 1782-1797, 1783-1798, 1784-1799, 1785-1800, 1822-1839, 1822-1837, 1823-1838, 1824-1839, 1866-1881, 1867-1882, 1868-1883, 1869-1884, 1870-1885, 1871-1886, 1872-1887, or 1874-1889, and wherein at least one nucleoside of the compound or modified oligonucleotide comprises at least one 2'-O-methoxyethyl or constrained ethyl (cEt) sugar.

In certain embodiments, an antisense compound or oligonucleotide targeted to a HBV nucleic acid is complementary within the following nucleotide regions of SEQ ID NO: 1: 58-73, 58-74, 58-77, 59-74, 59-75, 60-75, 60-76, 61-76, 61-77, 62-77, 253-272, 253-269, 254-270, 255-271, 256-272, 411-437, 411-426, 411-427, 411-430, 412-427, 412-428, 412-431, 413-428, 413-429, 413-432, 414-429, 414-430, 414-433, 415-430, 415-431, 415-434, 416-431, 416-432, 416-435, 417-432, 417-433, 417-436, 418-433, 418-434, 418-437, 457-472, 457-473, 458-473, 670-706, 670-685, 670-686, 671-686, 671-687, 672-687, 672-688, 673-688, 687-702, 687-703, 687-706, 688-703, 688-704, 689-704, 689-705, 690-705, 690-706, 691-706, 1261-1285, 1261-1276, 1261-1277, 1261-1280, 1262-1277, 1262-1278, 1262-1281, 1263-1278, 1263-1279, 1263-1282, 1264-1279, 1264-1280, 1264-1283, 1265-1280, 1265-1281, 1265-1284, 1266-1281, 1266-1282, 1266-1285, 1267-1282, 1267-1283, 1268-1283, 1268-1284, 1269-1284, 1269-1285, 1270-1285, 1577-1606, 1577-1592, 1577-1593, 1577-1596, 1578-1593, 1578-1594, 1578-1597, 1579-1594, 1579-1594, 1579-1598, 1580-1595, 1580-1596, 1580-1599, 1581-1596, 1581-1597, 1581-1600, 1582-1597, 1582-1598, 1582-1601, 1583-1598, 1583-1599, 1583-1602, 1584-1599, 1584-1600, 1584-1603, 1585-1600, 1585-1601, 1585-1604, 1586-1601, 1586-1602, 1586-1605, 1587-1602, 1587-1603, 1587-1606, 1588-1603, 1588-1604, 1589-1604, 1589-1605, 1590-1605, 1590-1606, 1591-1606, 1778-1800, 1778-1793, 1778-1794, 1778-1797, 1779-1794, 1779-1795, 1779-1798, 1780-1795, 1780-1796, 1780-1799, 1781-1796, 1781-1797, 1781-1800, 1782-1797, 1782-1798, 1783-1798, 1783-1799, 1784-1799, and 1784-1800.

In certain embodiments, an antisense compound or oligonucleotide targeted to a HBV nucleic acid target the following nucleotide regions of SEQ ID NO: 1: 58-73, 58-74, 58-77, 59-74, 59-75, 60-75, 60-76, 61-76, 61-77, 62-77, 253-272, 253-269, 254-270, 255-271, 256-272, 411-437, 411-426, 411-427, 411-430, 412-427, 412-428, 412-431, 413-428, 413-429, 413-432, 414-429, 414-430, 414-433, 415-430, 415-431, 415-434, 416-431, 416-432, 416-435, 417-432, 417-433, 417-436, 418-433, 418-434, 418-437, 457-472, 457-473, 458-473, 670-706, 670-685, 670-686, 671-686, 671-687, 672-687, 672-688, 673-688, 687-702, 687-703, 687-706, 688-703, 688-704, 689-704, 689-705, 690-705, 690-706, 691-706, 1261-1285, 1261-1276, 1261-1277, 1261-1280, 1262-1277, 1262-1278, 1262-1281, 1263-1278, 1263-1279, 1263-1282, 1264-1279, 1264-1280, 1264-1283, 1265-1280, 1265-1281, 1265-1284, 1266-1281, 1266-1282, 1266-1285, 1267-1282, 1267-1283, 1268-1283, 1268-1284, 1269-1284, 1269-1285, 1270-1285, 1577-1606, 1577-1592, 1577-1593, 1577-1596, 1578-1593, 1578-1594, 1578-1597, 1579-1594, 1579-1594, 1579-1598, 1580-1595, 1580-1596, 1580-1599, 1581-1596, 1581-1597, 1581-1600, 1582-1597, 1582-1598, 1582-1601, 1583-1598, 1583-1599, 1583-1602, 1584-1599, 1584-1600, 1584-1603, 1585-1600, 1585-1601, 1585-1604, 1586-1601, 1586-1602, 1586-1605, 1587-1602, 1587-1603, 1587-1606, 1588-1603, 1588-1604, 1589-1604, 1589-1605, 1590-1605, 1590-1606, 1591-1606, 1778-1800, 1778-1793, 1778-1794, 1778-1797, 1779-1794, 1779-1795, 1779-1798, 1780-1795, 1780-1796, 1780-1799, 1781-1796, 1781-1797, 1781-1800, 1782-1797, 1782-1798, 1783-1798, 1783-1799, 1784-1799, and 1784-1800.

In certain embodiments, antisense compounds or oligonucleotides target a region of a HBV nucleic acid. In certain embodiments, such compounds or oligonucleotides targeted to a region of a HBV nucleic acid have a contiguous nucleobase portion that is complementary to an equal length nucleobase portion of the region. For example, the portion can be at least an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobases portion complementary to an equal length portion of a region recited herein. In certain embodiments, such compounds or oligonucleotide target the following nucleotide regions of SEQ ID NO: 1: 1-20, 10-29, 10-56, 13-38, 13-35, 19-38, 25-47, 25-50, 25-56, 43-68, 43-63, 55-74, 58-73, 58-74, 58-77, 58-79, 58-80, 58-84, 59-74, 59-75, 59-80, 60-75, 60-76, 60-79, 61-76, 61-77, 61-80, 62-77, 63-84, 68-114, 101-123, 98-123, 113-138, 116-138, 131-150, 137-162, 152-186, 158-177, 167-186, 191-215, 196-224, 196-215, 196-218, 199-228, 199-218, 199-224, 200-224, 205-224, 206-228, 218-237, 224-243, 233-264, 242-263, 243-262, 244-263, 245-264, 246-266, 247-266, 247-269, 247-270, 245-267, 251-267, 245-266, 250-269, 251-268, 251-269, 245-269, 245-266, 245-261, 250-266, 250-267, 250-268, 250-269, 251-270, 253-269, 253-272, 253-274, 254-270, 254-274, 255-271, 255-274, 255-401, 255-400, 255-274, 255-273, 255-272, 255-271, 256-275, 255-276, 256-272, 256-276, 253-275, 256-279, 257-276, 260-279, 262-281, 262-321, 262-315, 262-312, 265-312, 266-288, 266-291, 266-285, 281-321, 281-303, 290-321, 290-312, 292-311, 290-312, 293-312, 293-315, 293-321, 296-321, 302-321, 324-343, 339-361, 339-367, 348-367, 342-367, 358-392, 358-378, 360-392, 360-383, 360-388, 360-385, 362-381, 366-388, 369-388, 366-385, 366-392, 370-389, 370-392, 384-433, 384-400, 384-401, 385-401, 405-424, 409-428, 405-428, 411-426, 411-427, 411-430, 411-431, 411-437, 412-431, 411-

426, 411-427, 412-428, 412-431, 412-427, 413-433, 413-432, 413-428, 413-429, 413-432, 413-433, 414-427, 415-427, 414-429, 414-430, 414-433, 415-428, 415-429, 415-430, 415-431, 415-434, 416-431, 416-432, 416-429, 416-435, 417-432, 417-433, 417-436, 418-433, 418-435, 418-434, 418-437, 419-435, 419-434, 420-435, 419-432, 419-434, 422-441, 423-436, 425-465, 454-473, 454-472, 457-476, 457-472, 457-473, 454-476, 455-472, 457-485, 458-485, 458-483, 458-477, 458-473, 459-485, 460-485, 463-498, 463-485, 466-485, 463-482, 457-491, 458-491, 459-491, 460-491, 463-491, 466-491, 472-491, 472-493, 473-492, 475-491, 459-494, 460-494, 463-494, 466-494, 467-498, 472-494, 475-494, 457-473, 457-472, 458-494, 454-494, 457-494, 457-473, 485-513, 470-493, 476-519, 485-519, 500-519, 512-534, 512-550, 524-546, 536-559, 548-567, 548-570, 550-570, 548-594, 554-573, 548-576, 560-594, 584-606, 611-645, 617-363, 623-642, 617-645, 642-754, 653-672, 662-685, 665-685, 665-689, 668-687, 670-706, 670-685, 670-686, 670-689, 671-690, 671-691, 671-686, 671-687, 672-693, 672-697, 672-707, 672-687, 672-688, 673-688, 674-693, 679-707, 679-698, 679-701, 679-702, 679-707, 680-699, 679-699, 682-706, 682-707, 682-702, 682-701, 687-754, 688-704, 689-709, 689-710, 690-705, 679-705, 679-710, 679-706, 690-710, 691-710, 690-754, 690-706, 684-703, 687-705, 687-702, 687-703, 687-706, 688-703, 688-704, 688-705, 689-704, 689-705, 689-708, 690-705, 690-706, 690-709, 691-706, 692-711, 693-716, 693-712, 695-715, 697-716, 697-716, 690-716, 724-746, 724-752, 724-754, 724-758, 733-752, 738-754, 739-758, 739-754, 739-775, 739-754, 740-754, 742-785, 742-773, 757-776, 757-785, 790-815, 793-812, 811-833, 811-844, 814-833, 811-906, 820-839, 822-844, 822-867, 823-842, 845-864, 845-867, 854-906, 845-909, 845-906, 854-876, 863-882, 863-885, 878-900, 887-906, 899-918, 899-933, 899-958, 905-927, 905-933, 914-933, 936-958, 936-955, 945-964, 951-970, 951-985, 951-1044, 951-1024, 951-1056, 951-997, 960-985, 963-1044, 963-1024, 963-997, 972-1015, 1025-1044, 1031-1056, 1037-1056, 1046-1083, 1049-1068, 1070-1089, 1070-1095, 1082-1101, 1081-1134, 1081-1143, 1082-1101, 1088-1107, 1088-1134, 1094-1119, 1097-1119, 1112-1134, 1118-1143, 1118-1146, 1088-1146, 1121-1140, 1127-1146, 1127-1193, 1150-1193, 1156-1187, 1165-1187, 1170-1192, 1171-1191, 1172-1191, 1176-1192, 1177-1192, 1176-1191, 1203-1297, 1206-1228, 1206-1255, 1209-1228, 1215-1255, 1245-1265, 1251-1280, 1262-1285, 1251-1285, 1259-1296, 1259-1290, 1259-1287, 1261-1296, 1261-1285, 1261-1276, 1261-1277, 1261-1280, 1262-1296, 1262-1277, 1262-1278, 1262-1281, 1263-1278, 1263-1279, 1263-1282, 1264-1279, 1264-1280, 1264-1283, 1264-1297, 1265-1280, 1265-1281, 1265-1284, 1266-1281, 1266-1282, 1266-1285, 1267-1282, 1267-1283, 1268-1283, 1268-1284, 1268-1296, 1269-1284, 1269-1285, 1269-1288, 1270-1285, 1271-1290, 1271-1296, 1277-1296, 1261-1290, 1262-1290, 1268-1290, 1263-1305, 1259-1305, 1259-1305, 1266-1305, 1259-1302, 1275-1294, 1281-1306, 1281-1324, 1281-1336, 1282-1301, 1286-1306, 1290-1324, 1293-1318, 1290-1324, 1293-1315, 1296-1315, 1311-1336, 1311-1333, 1326-1345, 1353-1381, 1359-1378, 1395-1414, 1498-1532, 1498-1523, 1498-1535, 1510-1529, 1515-1535, 1515-1563, 1515-1596, 1515-1605, 1515-1602, 1515-1540, 1515-1535, 1518-1605, 1518-1602, 1518-1537, 1521-1563, 1521-1540, 1550-1655, 1550-1563, 1550-1569, 1553-1578, 1553-1599, 1553-1590, 1565-1584, 1571-1595, 1577-1605, 1577-1606, 1577-1592, 1577-1593, 1577-1596, 1578-1593, 1578-1594, 1578-1597, 1578-1598, 1571-1598, 1579-1594, 1579-1594, 1579-1598, 1580-1595,

1580-1596, 1580-1599, 1580-1605, 1580-1602, 1581-1596, 1581-1597, 1581-1600, 1582-1597, 1582-1598, 1582-1601, 1582-1602, 1553-1655, 1583-1598, 1583-1599, 1583-1602, 1584-1599, 1584-1600, 1584-1603, 1585-1600, 1585-1601, 1585-1604, 1586-1601, 1586-1602, 1586-1605, 1587-1602, 1587-1603, 1587-1606, 1588-1603, 1588-1604, 1589-1604, 1589-1605, 1590-1605, 1590-1606, 1591-1606, 1586-1652, 1642-1664, 1651-1720, 1651-1673, 1655-1679, 1695-1720, 1716-1738, 1743-1763, 1743-1768, 1764-1783, 1773-1792, 1777-1796, 1777-1800, 1778-1800, 1778-1793, 1778-1794, 1778-1797, 1779-1798, 1779-1797, 1779-1796, 1779-1795, 1779-1794, 1780-1799, 1780-1796, 1780-1795, 1781-1797, 1781-1796, 1781-1796, 1781-1800, 1781-1797, 1782-1799, 1782-1797, 1782-1798, 1783-1798, 1783-1799, 1784-1800, 1784-1799, 1779-1799, 1778-1794, 1779-1795, 1780-1799, 1794-1813, 1806-1837, 1806-1828, 1806-1825, 1809-1828, 1812-1843, 1812-1837, 1812-1831, 1815-1843, 1815-1844, 1815-1840, 1815-1834, 1818-1837, 1821-1840, 1821-1844, 1821-1837, 1822-1843, 1822-1839, 1823-1843, 1827-1846, 1861-1884, 1861-1880, 1865-1885, 1867-1886, 1869-1885, 1876-1895, 1888-1914, 1888-1908, 1891-1910, 1891-1914, 1895-1938, 1895-1935, 1913-1935, 1898-1920, 1907-1929, 1913-1935, 1918-1934, 1919-1938, 1919-1934, 1921-1934, 1928-1956, 1957-1976, 2035-2057, 2083-2141, 2230-2261, 2368-2393, 2381-2397, 2368-2394, 2379-2394, 2381-2396, 2368-2397, 2368-2396, 2420-2439, 2458-2476, 2459-2478, 2819-2838, 2818-2838, 2873-2892, and 3161-3182.

In certain embodiments, antisense compounds or oligonucleotides target a region of a HBV nucleic acid. In certain embodiments, such compounds or oligonucleotides targeted to a region of a HBV nucleic acid have a contiguous nucleobase portion that is complementary to an equal length nucleobase portion of the region. For example, the portion can be at least an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobases portion complementary to an equal length portion of a region recited herein. In certain embodiments, such compounds or oligonucleotide target the following nucleotide regions of SEQ ID NO: 1: 233-264, 242-263, 243-262, 244-263, 245-264, 246-266, 247-266, 247-269, 247-270, 245-267, 251-267, 245-266, 250-269, 251-268, 251-269, 245-269, 245-266, 245-261, 250-266, 250-267, 250-268, 250-269, 251-270, 253-272, 253-274, 254-274, 255-274, 255-401, 255-400, 255-274, 255-273, 255-272, 255-271, 256-275, 255-276, 256-276, 253-275, 256-279, 257-276, 260-279, 262-281, 262-321, 262-315, 262-312, 265-312, 266-288, 266-291, 266-285, 281-321, 281-303, 405-424, 409-428, 405-428, 411-430, 411-431, 411-431, 412-431, 411-426, 411-427, 412-428, 412-431, 412-427, 413-433, 413-432, 413-428, 413-433, 411-427, 414-427, 415-427, 415-428, 415-429, 416-432, 416-429, 418-435, 418-434, 419-435, 419-434, 420-435, 419-432, 419-434, 422-441, 423-436, 425-465, 584-606, 611-645, 617-363, 623-642, 617-645, 642-754, 653-672, and wherein at least one nucleoside of the compound or modified oligonucleotide comprises at least one 2'-O-methoxyethyl sugar.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 50% inhibition: 1-20, 10-29, 10-56, 13-38, 13-35, 19-38, 25-47, 25-50, 25-56, 43-68, 43-63, 55-74, 58-77, 58-74, 58-73, 58-79, 58-80, 58-84, 59-74, 59-75,

59-80, 60-79, 60-75, 60-76, 61-80, 61-76, 61-77, 62-77, 63-84, 68-114, 101-123, 98-123, 113-138, 116-138, 131-150, 137-162, 152-186, 158-177, 167-186, 191-215, 196-224, 196-215, 196-218, 199-228, 199-218, 199-224, 200-224, 205-224, 206-228, 218-237, 224-243, 233-264, 242-263, 243-262, 244-263, 245-264, 246-266, 247-266, 247-269, 247-270, 245-267, 251-267, 245-266, 250-269, 251-268, 251-269, 245-269, 245-266, 245-261, 250-265, 250-266, 250-267, 250-268, 250-269, 251-266, 251-270, 252-267, 253-268, 253-269, 253-272, 253-274, 254-269, 254-270, 254-274, 255-274, 255-401, 255-400, 255-274, 255-273, 255-272, 255-271, 255-270, 256-271, 256-272, 256-275, 255-276, 256-276, 253-275, 256-279, 257-276, 258-273, 259-274, 260-279, 262-281, 262-321, 262-315, 262-312, 265-312, 266-288, 266-291, 266-285, 281-321, 281-303, 290-321, 290-312, 292-311, 290-312, 293-312, 293-315, 293-321, 296-321, 302-321, 324-343, 339-361, 339-367, 348-367, 342-367, 358-392, 358-378, 360-392, 360-383, 360-388, 360-385, 362-381, 366-388, 369-388, 366-385, 366-392, 370-389, 370-392, 380-399, 382-401, 384-433, 384-400, 384-401, 385-401, 405-424, 409-428, 405-428, 411-430, 411-431, 411-431, 412-431, 411-426, 411-427, 411-430, 411-437, 412-428, 412-431, 412-427, 413-432, 413-428, 413-429, 413-433, 411-427, 414-427, 414-429, 414-430, 414-433, 415-427, 415-428, 415-429, 415-430, 415-431, 415-434, 416-435, 416-432, 416-431, 416-429, 417-432, 417-433, 417-436, 418-437, 418-435, 418-434, 418-433, 419-435, 419-434, 420-435, 419-432, 419-434, 421-436, 422-441, 422-437, 423-436, 425-465, 454-473, 454-472, 457-476, 454-476, 455-472, 457-485, 457-473, 457-472, 458-485, 458-483, 458-477, 458-473, 459-485, 460-485, 463-498, 463-485, 466-485, 463-482, 457-491, 458-491, 459-491, 460-491, 463-491, 466-491, 472-491, 472-493, 473-492, 475-491, 459-494, 460-494, 463-494, 466-494, 467-498, 472-494, 475-494, 457-473, 457-472, 458-494, 454-494, 457-494, 457-473, 485-513, 470-493, 476-519, 485-519, 500-519, 512-534, 512-550, 524-546, 536-559, 548-567, 548-570, 550-570, 548-594, 554-573, 548-576, 560-594, 584-606, 611-645, 617-363, 623-642, 617-645, 639-654, 641-656, 642-657, 642-754, 643-658, 653-672, 662-685, 665-685,665-689, 668-687, 670-689, 670-706, 670-685, 670-686, 671-686, 671-687, 671-690, 671-691, 672-687, 672-688, 672-693, 672-697, 672-707, 673-688, 674-693, 678-693, 679-707, 679-694, 679-698, 679-701, 679-702, 679-707, 680-699, 679-699, 680-695, 681-696, 682-706, 682-707, 682-702, 682-701, 682-697, 683-698, 684-699, 685-700, 686-701, 687-702, 687-703, 687-706, 687-754, 688-703, 688-704, 689-704, 689-705, 689-709, 689-710, 690-705, 690-706, 691-706, 679-705, 679-710, 679-706, 690-710, 691-710, 690-754, 690-706, 684-703, 687-705, 687-703, 687-706, 688-705, 689-708, 690-709, 692-711, 693-716, 693-712, 695-715, 697-716, 697-716, 690-716, 724-746, 724-752, 724-754, 724-758, 733-752, 738-753, 738-754, 739-758, 739-754, 739-775, 739-754, 740-754, 742-785, 742-773, 757-776, 757-785, 790-815, 793-812, 811-833, 811-844, 814-833, 811-906, 820-839, 822-844, 822-867, 823-842, 845-864, 845-867, 854-906, 845-909, 845-906, 854-876, 854-873, 863-882, 863-885, 878-900, 878-897, 887-906, 899-918, 899-933, 899-958, 905-927, 905-933, 914-933, 936-958, 936-955, 945-964, 951-970, 951-985, 951-1044, 951-1024, 951-1056, 951-997, 960-985, 963-1044, 963-1024, 963-997, 966-985, 972-1015, 978-997, 1025-1044, 1031-1056, 1037-1056, 1046-1083, 1049-1068, 1070-1089, 1070-1095, 1082-1101, 1081-1134, 1081-1143, 1082-1101, 1088-1107, 1088-1134, 1094-1119, 1097-1119, 1112-1134, 1118-1143, 1118-1146, 1088-1146, 1121-1140,

1127-1146, 1127-1193, 1150-1193, 1156-1187, 1165-1187, 1170-1192, 1171-1191, 1172-1191, 1176-1192, 1177-1192, 1176-1191, 1203-1297, 1206-1228, 1206-1255, 1209-1228, 1215-1255, 1215-1234, 1218-1237, 1221-1240, 1224-1243, 1227-1246, 1230-1249, 1233-1252, 1236-1255, 1245-1265, 1251-1280, 1251-1285, 1251-1270, 1254-1273, 1254-1279, 1257-1276, 1258-1277, 1259-1296, 1259-1290, 1259-1287, 1260-1279, 1261-1285, 1261-1276, 1261-1277, 1261-1280, 1261-1296, 1262-1277, 1262-1278, 1262-1281, 1262-1285, 1262-1296, 1263-1278, 1263-1279, 1263-1282, 1264-1279, 1264-1280, 1264-1283, 1264-1297, 1265-1280, 1265-1281, 1265-1284, 1266-1281, 1266-1282, 1266-1285, 1267-1282, 1267-1283, 1268-1283, 1268-1284, 1268-1296, 1269-1284, 1269-1285, 1269-1288, 1270-1285, 1271-1290, 1271-1296, 1277-1296, 1261-1290, 1262-1290, 1268-1290, 1263-1305, 1259-1305, 1259-1305, 1266-1305, 1259-1302, 1275-1294, 1281-1306, 1281-1324, 1281-1336, 1782-1797, 1282-1301, 1286-1306, 1290-1324, 1293-1318, 1290-1324, 1293-1315, 1296-1315, 1311-1336, 1311-1333, 1326-1345, 1353-1381, 1359-1378, 1395-1414, 1498-1532, 1498-1523, 1498-1535, 1510-1529, 1515-1535, 1515-1563, 1515-1596, 1515-1605, 1515-1602, 1515-1540, 1515-1535, 1518-1605, 1518-1602, 1518-1537, 1521-1563, 1521-1540, 1550-1655, 1550-1563, 1550-1569, 1553-1578, 1553-1599, 1553-1590, 1565-1584, 1571-1595, 1577-1606, 1577-1605, 1577-1596, 1577-1592, 1577-1593, 1578-1593, 1578-1594, 1578-1597, 1578-1598, 1579-1594, 1579-1595, 1579-1598, 1571-1598, 1580-1605, 1580-1602, 1580-1595, 1580-1596, 1580-1599, 1581-1596, 1581-1597, 1581-1600, 1582-1597, 1582-1598, 1582-1601, 1582-1602, 1553-1655, 1583-1598, 1583-1599, 1583-1602, 1584-1599, 1584-1600, 1584-1603, 1585-1600, 1585-1601, 1585-1604, 1586-1601, 1586-1602, 1586-1605, 1586-1652, 1587-1602, 1587-1603, 1587-1606, 1588-1603, 1588-1604, 1589-1604, 1589-1605, 1590-1605, 1590-1606, 1591-1606, 1642-1664, 1651-1720, 1651-1673, 1655-1679, 1695-1720, 1716-1738, 1743-1763, 1743-1768, 1764-1783, 1773-1792, 1777-1796, 1777-1800, 1778-1800, 1778-1793, 1778-1794, 1778-1797, 1779-1798, 1779-1797, 1779-1796, 1779-1795, 1779-1794, 1780-1796, 1781-1797, 1781-1796, 1781-1800, 1781-1797, 1782-1799, 1784-1800, 1779-1799, 1778-1794, 1779-1795, 1780-1799, 1794-1813, 1780-1795, 1780-1796, 1780-1799, 1781-1796, 1781-1797, 1781-1800, 1782-1798, 1783-1799, 1784-1799, 1784-1800, 1785-1800, 1806-1837, 1806-1828, 1806-1825, 1809-1828, 1812-1843, 1812-1837, 1812-1831, 1815-1843, 1815-1844, 1815-1840, 1815-1834, 1818-1837, 1821-1840, 1821-1844, 1821-1837, 1822-1843, 1822-1839, 1823-1843, 1827-1846, 1861-1884, 1861-1880, 1865-1885, 1867-1886, 1869-1885, 1876-1895, 1888-1914, 1888-1908, 1891-1910, 1891-1914, 1895-1938, 1895-1935, 1913-1935, 1898-1920, 1907-1929, 1913-1935, 1918-1934, 1919-1938, 1919-1934, 1921-1934, 1928-1956, 1957-1976, 2035-2057, 2083-2141, 2230-2261, 2278-2297, 2281-2300, 2284-2303, 2368-2393, 2381-2397, 2368-2394, 2379-2394, 2381-2396, 2368-2397, 2368-2396, 2420-2439, 2458-2476, 2459-2478, 2819-2838, 2818-2838, 2873-2892, and 3161-3182.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 60% inhibition: 1-20, 10-29, 10-53, 13-38, 25-50, 43-68, 55-74, 58-84, 58-77, 58-74, 58-73, 58-79, 59-80, 59-74, 59-75, 60-75, 60-76, 61-77, 61-76, 61-80, 62-77, 68-114, 98-123, 101-123, 113-138, 116-138, 131-150, 137-162, 152-186, 191-215, 196-224, 196-215,

199-228, 199-218, 200-223, 199-218, 205-224, 206-228, 218-237, 224-243, 233-263, 244-263, 245-264, 247-266, 250-265, 251-266, 252-267, 253-272, 253-269, 251-267, 253-274, 254-270, 255-276, 256-279, 256-276, 256-274, 256-272, 256-271, 258-273, 259-274, 265-388, 265-284, 266-291, 266-288, 260-279, 281-321, 281-303, 290-321, 290-312, 293-312, 296-315, 302-321, 324-343, 339-367, 339-361, 342-367, 348-367, 358-392, 358-378, 360-392, 360-379, 366-392, 366-385, 369-388, 370-392, 382-401, 405-428, 405-424, 409-428, 411-436, 411-433, 411-431, 411-426, 411-430, 411-427, 412-431, 412-428, 412-427, 413-428, 413-429, 413-433, 414-433, 414-430, 414-429, 414-433, 415-430, 415-431, 415-434, 415-435, 415-436, 416-429, 416-434, 416-431, 416-432, 416-436, 416-435, 417-436, 417-433, 417-432, 418-434, 418-433, 418-437, 419-434, 420-435, 421-436, 422-437, 423-436, 425-465, 454-472, 455-472, 457-476, 457-472, 457-473, 458-485, 458-473, 458-483, 463-498, 467-498, 463-482, 470-493, 472-491, 485-519, 485-513, 500-519, 512-534, 524-546, 536-558, 548-567, 554-573, 548-576, 560-594, 584-606, 608-648, 639-654, 640-656, 641-656, 642-657, 642-658, 643-658, 653-672, 662-685, 665-685, 670-706, 670-689, 670-685, 670-686, 671-690, 671-686, 671-687, 672-707, 672-697, 672-693, 672-687, 672-688, 673-688, 679-707, 679-698, 679-694, 680-695, 681-696, 682-697, 682-701, 683-698, 684-699, 685-700, 686-701, 687-754, 687-702, 687-705, 687-703, 687-706, 688-704, 688-703, 688-704, 688-705, 688-707, 689-710, 689-709, 689-705, 689-704, 690-754, 690-705, 690-706, 691-706, 691-710, 692-711, 697-716, 724-758, 724-754, 724-752, 724-746, 738-754, 738-753, 739-754, 742-785, 757-785, 790-815, 811-906, 811-844, 811-833, 822-867, 822-844, 823-842, 845-867, 854-906, 854-873, 878-897, 899-958, 899-933, 936-958, 945-964, 951-1044, 951-1024, 951-985, 951-997, 963-1044, 963-1024, 963-997, 966-985, 978-997, 1031-1056, 1046-1083, 1070-1095, 1081-1143, 1081-1134, 1082-1101, 1088-1146, 1088-1134, 1118-1146, 1118-1143, 1127-1193, 1170-1189, 1176-1192, 1176-1191, 1177-1192, 1203-1297, 1206-1255, 1209-1228, 1215-1234, 1218-1237, 1221-1240, 1224-1243, 1227-1246, 1230-1249, 1233-1252, 1236-1255, 1251-1270, 1251-1285, 1254-1273, 1254-1279, 1257-1276, 1258-1277, 1259-1278, 1260-1279, 1261-1276, 1261-1285, 1261-1276, 1261-1277, 1261-1280, 1262-1281, 1262-1277, 1262-1278, 1262-1281, 1263-1278, 1263-1279, 1263-1282, 1264-1297, 1264-1279, 1264-1280, 1264-1283, 1265-1280, 1265-1281, 1265-1284, 1266-1281, 1266-1282, 1266-1285, 1267-1282, 1267-1283, 1268-1283, 1268-1284, 1268-1287, 1269-1284, 1269-1285, 1270-1285, 1281-1336, 1281-1324, 1281-1306, 1286-1305, 1290-1324, 1311-1336, 1326-1345, 1353-1381, 1395-1414, 1498-1535, 1498-1532, 1515-1535, 1515-1534, 1521-1540, 1550-1655, 1553-1599, 1553-1590, 1577-1606, 1577-1592, 1577-1593, 1577-1596, 1578-1593, 1578-1594, 1578-1597, 1579-1594, 1579-1595, 1579-1598, 1580-1595, 1580-1596, 1580-1599, 1581-1596, 1581-1597, 1581-1600, 1582-1597, 1582-1598, 1582-1601, 1583-1598, 1583-1599, 1583-1602, 1584-1599, 1584-1600, 1584-1603, 1585-1600, 1585-1601, 1585-1604, 1586-1601, 1586-1602, 1586-1605, 1587-1602, 1587-1603, 1587-1606, 1588-1603, 1588-1604, 1589-1604, 1589-1605, 1590-1605, 1590-1606, 1591-1606, 1642-1664, 1651-1720, 1716-1738, 1743-1763, 1764-1783, 1773-1792, 1777-1800, 1777-1797, 1655-1674, 1778-1794, 1778-1800, 1781-1800, 1781-1797, 1784-1800, 1779-1799, 1778-1794, 1778-1797, 1779-1795, 1779-1798, 1780-1795, 1780-1796, 1780-1799, 1781-1796, 1781-1797, 1781-1800, 1782-1797, 1794-1813, 1806-1837, 1806-1825, 1812-

1837, 1812-1831, 1815-1844, 1815-1834, 1818-1837, 1821-1837, 1822-1838, 1827-1846, 1861-1884, 1821-1840, 1866-1885, 1867-1886, 1888-1914, 1888-1907, 1891-1914, 1895-1938, 1895-1935, 1919-1938, 1928-1956, 1957-1976, 2035-2057, 2083-2141, 2230-2261, 2278-2297, 2281-2300, 2284-2303, 2368-2397, 2368-2396, 2368-2394, 2368-2393, 2379-2394, 2381-2396, 2420-2439, 2458-2476, 2819-2838, 2873-2892, and 3161-3182.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 65% inhibition: 1-20, 10-29, 10-53, 13-38, 25-50, 43-68, 55-74, 58-84, 58-79, 58-74, 58-73, 58-77, 59-75, 59-80, 58-77, 60-75, 60-76, 61-77, 61-76, 61-80, 62-77, 68-114, 98-123, 101-123, 113-138, 116-138, 131-150, 137-162, 152-186, 191-215, 196-215, 199-228, 199-218, 200-223,199-218, 205-224, 206-228, 218-237, 224-243, 233-263, 244-263, 245-264, 250-265, 251-266, 253-269, 253-274, 255-276, 256-279, 256-276, 256-274, 256-272, 256-271, 247-266, 253-272, 258-273, 266-291, 266-288, 260-279, 281-321, 281-303, 290-321, 290-312, 296-315, 293-312, 302-321, 324-343, 339-367, 339-361, 342-367, 348-367, 358-392, 358-378, 360-392, 360-379, 366-392, 366-385, 369-388, 370-392, 382-401, 405-428, 405-424, 409-428, 411-433, 411-431, 411-430, 411-427, 411-426, 412-431, 412-428, 412-427, 413-433, 413-428, 413-429, 413-432, 414-433, 414-430, 414-429, 415-430, 415-431, 415-434, 415-435, 415-436, 416-434, 416-436, 416-435, 416-432, 416-431, 417-436, 417-433, 417-432, 418-433, 418-434, 418-437, 420-435, 422-437, 423-436, 425-465, 454-472, 455-472, 457-472, 458-485, 458-483, 458-473, 463-498, 467-498, 457-476, 470-493, 472-491, 485-519, 485-513, 500-519, 512-534, 524-546, 536-558, 548-567, 554-573, 548-576, 560-594, 584-606, 608-648, 639-654, 640-656, 641-656, 642-657, 642-658, 643-658, 653-672, 662-685, 665-685, 670-685, 670-706, 670-689, 670-686, 670-685, 671-686, 671-687, 671-690, 672-688, 672-687, 672-707, 672-697, 672-693, 673-688, 679-698, 680-695, 681-696, 682-697, 682-701, 683-698, 684-699, 685-700, 686-701, 687-702, 688-703, 688-707, 687-754, 690-754, 690-706, 690-705, 687-705, 687-703, 687-706, 687-702, 688-705, 688-703, 688-704, 689-705, 691-706, 692-711, 697-716, 724-758, 724-754, 724-752, 724-746, 738-754, 739-754, 742-785, 757-785, 790-815, 811-906, 811-844, 811-833, 822-867, 822-844, 823-842, 845-867, 854-906, 854-873, 878-897, 899-958, 899-933, 936-958, 945-964, 951-1044, 951-1024, 951-985, 951-997, 963-1044, 963-1024, 963-997, 966-985, 978-997, 1031-1056, 1046-1083, 1070-1095, 1081-1143, 1081-1134, 1082-1101, 1088-1146, 1088-1134, 1118-1146, 1118-1143, 1127-1193, 1170-1189, 1176-1192, 1177-1192, 1203-1297, 1206-1255, 1209-1228, 1215-1234, 1218-1237, 1221-1240, 1224-1243, 1227-1246, 1230-1249, 1233-1252, 1236-1255, 1251-1270, 1251-1285, 1254-1273, 1254-1279, 1257-1276, 1258-1277, 1259-1278, 1260-1279, 1261-1285, 1261-1276, 1261-1277, 1261-1280, 1262-1281, 1262-1277, 1262-1278, 1263-1278, 1263-1279, 1263-1282, 1264-1297, 1264-1279, 1264-1280, 1264-1283, 1265-1280, 1265-1281, 1265-1284, 1266-1281, 1266-1282, 1266-1285, 1267-1282, 1267-1283, 1268-1283, 1268-1284, 1268-1287, 1269-1284, 1269-1285, 1270-1285, 1281-1336, 1281-1324, 1281-1306, 1290-1324, 1311-1336, 1326-1345, 1353-1381, 1395-1414, 1498-1535, 1498-1532, 1515-1535, 1515-1534, 1550-1655, 1553-1599, 1553-1590, 1577-1606, 1577-1592, 1577-1593, 1577-1596, 1578-1593, 1578-1594, 1578-1597, 1579-1594, 1579-

1595, 1579-1598, 1580-1595, 1580-1596, 1580-1599, 1581-1596, 1581-1597, 1581-1600, 1582-1597, 1582-1598, 1582-1601, 1583-1598, 1583-1599, 1583-1602, 1584-1599, 1584-1600, 1584-1603, 1585-1600, 1585-1601, 1585-1604, 1586-1601, 1586-1602, 1586-1605, 1587-1602, 1587-1603, 1587-1606, 1588-1603, 1588-1604, 1589-1604, 1589-1605, 1590-1605, 1590-1606, 1591-1606, 1642-1664, 1651-1720, 1655-1674, 1716-1738, 1743-1763, 1764-1783, 1773-1792, 1777-1800, 1777-1797, 1778-1800, 1778-1797, 1779-1799, 1778-1794, 1779-1794, 1779-1795, 1779-1798, 1780-1796, 1780-1799, 1780-1795, 1781-1796, 1781-1797, 1781-1800, 1782-1797, 1794-1813, 1806-1837, 1806-1825, 1812-1837, 1812-1831, 1815-1844, 1815-1834, 1818-1837, 1821-1837, 1822-1838, 1827-1846, 1861-1884, 1866-1885, 1867-1886, 1888-1914, 1888-1907, 1891-1914, 1895-1938, 1895-1935, 1919-1938, 1928-1956, 1957-1976, 2035-2057, 2083-2141, 2230-2261, 2278-2297, 2281-2300, 2284-2303, 2368-2397, 2368-2396, 2368-2394, 2368-2393, 2379-2394, 2381-2396, 2420-2439, 2458-2476, 2819-2838, 2873-2892, and 3161-3182.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 70% inhibition: 1-20, 10-29, 10-53, 13-38, 25-50, 43-68, 55-74, 58-84, 58-79, 58-74, 59-75, 59-80, 58-77, 60-75, 60-76, 61-77, 68-114, 98-123, 101-123, 113-138, 116-138, 131-150, 137-162, 152-186, 191-215, 199-228, 199-218, 200-223, 205-224, 206-228, 218-237, 224-243, 233-263, 244-263, 245-264, 253-269, 253-274, 255-276, 256-279, 256-276, 256-274, 256-272, 247-266, 250-265, 251-266, 253-272, 256-271, 266-291, 266-288, 260-279, 281-321, 281-303, 290-321, 290-312, 293-312, 302-321, 324-343, 339-367, 339-361, 342-367, 348-367, 358-392, 358-378, 360-392, 360-379, 366-392, 366-385, 370-392, 382-401, 405-428, 405-424, 409-428, 411-433, 411-431, 411-430, 411-427, 411-426, 412-431, 412-428, 412-427, 413-428, 413-429, 413-432, 414-433, 414-430, 414-429, 415-430, 414-433, 415-434, 415-435, 415-436, 416-431, 416-434, 416-436, 416-435, 416-432, 417-436, 417-433, 418-433, 418-437, 423-436, 425-465, 454-472, 455-472, 457-472, 457-476, 458-473, 458-485, 458-483, 463-498, 467-498, 457-476, 470-493, 470-493, 472-491, 485-519, 485-513, 485-519, 485-513, 500-519, 512-534, 524-546, 536-558, 548-567, 554-573, 548-576, 560-594, 584-606, 608-648, 639-654, 640-656, 641-656, 642-657, 642-658, 643-658, 653-672, 662-685, 665-685, 670-706, 670-689, 670-685, 670-686, 671-690, 671-686, 671-687, 672-687, 672-688, 672-707, 672-697, 672-693, 673-688, 679-698, 681-696, 682-697, 682-701, 683-698, 684-699, 686-701, 687-702, 687-754, 687-702, 688-703, 690-754, 690-706, 687-705, 687-703, 687-706, 692-711, 697-716, 724-758, 724-754, 724-752, 724-746, 738-754, 739-754, 738-754, 742-785, 757-785, 790-815, 811-906, 811-844, 811-833, 822-867, 822-844, 845-867, 854-906, 854-873, 878-897, 899-958, 899-933, 936-958, 945-964, 951-1044, 951-1024, 951-985, 951-997, 963-1044, 963-1024, 963-997, 966-985, 978-997, 1031-1056, 1046-1083, 1070-1095, 1081-1143, 1081-1134, 1082-1101, 1088-1146, 1088-1134, 1118-1146, 1118-1143, 1127-1193, 1170-1189, 1176-1192, 1177-1192, 1203-1297, 1206-1255, 1209-1228, 1215-1234, 1218-1237, 1221-1240, 1224-1243, 1227-1246, 1230-1249, 1233-1252, 1236-1255, 1251-1285, 1251-1270, 1254-1273, 1254-1279, 1257-1276, 1258-1277, 1259-1278, 1260-1279, 1261-1285, 1261-1276, 1261-1277, 1261-1280, 1262-1281, 1262-1277, 1262-1278, 1263-1278, 1263-1279, 1263-1282, 1264-1297, 1264-1279, 1264-1280, 1264-

1283, 1265-1281, 1265-1284, 1266-1282, 1266-1285, 1267-1282, 1267-1283, 1268-1283, 1268-1284, 1268-1287, 1269-1284, 1269-1285, 1270-1285, 1281-1336, 1281-1324, 1281-1306, 1290-1324, 1311-1336, 1326-1345, 1353-1381, 1395-1414, 1498-1535, 1498-1532, 1515-1535, 1550-1655, 1553-1599, 1553-1590, 1577-1606, 1577-1592, 1577-1593, 1577-1596, 1578-1593, 1578-1594, 1578-1597, 1579-1594, 1579-1595, 1579-1598, 1580-1595, 1580-1596, 1580-1599, 1581-1596, 1581-1597, 1581-1600, 1582-1597, 1582-1598, 1582-1601, 1583-1598, 1583-1599, 1583-1602, 1584-1599, 1584-1600, 1584-1603, 1585-1600, 1585-1601, 1585-1604, 1586-1602, 1586-1605, 1587-1602, 1587-1603, 1587-1606, 1588-1603, 1588-1604, 1589-1604, 1589-1605, 1590-1605, 1590-1606, 1591-1606, 1642-1664, 1651-1720, 1716-1738, 1743-1763, 1764-1783, 1773-1792, 1777-1800, 1777-1797, 1778-1800, 1778-1797, 1779-1799, 1778-1794, 1779-1795, 1779-1798, 1780-1795, 1780-1796, 1780-1799, 1781-1800, 1782-1797, 1794-1813, 1806-1837, 1806-1825, 1812-1837, 1812-1831, 1815-1844, 1815-1834, 1818-1837, 1821-1837, 1822-1838, 1827-1846, 1861-1884, 1866-1885, 1867-1886, 1888-1914, 1888-1907, 1891-1914, 1895-1938, 1895-1935, 1919-1938, 1928-1956, 1957-1976, 2035-2057, 2083-2141, 2230-2261, 2278-2297, 2281-2300, 2284-2303, 2368-2397, 2368-2396, 2368-2394, 2368-2393, 2379-2394, 2381-2396, 2420-2439, 2458-2476, 2819-2838, 2873-2892, and 3161-3182.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 75% inhibition: 13-32, 16-35, 19-38, 25-44, 28-47, 31-50, 43-62, 46-65, 49-68, 55-74, 58-82, 58-74, 58-77, 59-75, 60-75, 60-76, 61-77, 65-84, 98-117, 101-120, 104-123, 116-135, 119-138, 131-150, 137-156, 140-159, 143-162, 158-177, 161-180, 164-183, 167-186, 200-219, 203-226, 209-228, 218-237, 233-252, 236-255, 239-258, 242-264, 247-266, 251-266, 253-272, 255-276, 266-285, 269-288, 281-300, 284-303, 290-313, 298-317, 302-321, 324-343, 339-358, 342-361, 348-367, 358-381, 364-383, 366-386, 370-389, 373-392, 382-401, 405-424, 409-428, 411-430, 411-426, 411-427, 412-427, 412-431, 413-428, 413-429, 413-432, 414-436, 414-430, 414-429, 415-430, 416-431, 416-432, 417-433, 418-437, 422-441, 425-444, 428-447, 434-453, 440-459, 443-462, 446-465, 456-477, 458-473, 464-483, 470-493, 476-495, 479-498, 488-507, 491-510, 494-513, 500-519, 512-531, 515-534, 524-543, 527-546, 536-555, 539-558, 560-579, 566-585, 569-588, 572-591, 575-594, 584-603, 587-606, 608-627, 614-633, 617-636, 620-639, 623-642, 626-645, 629-648, 639-654, 641-656, 642-657, 643-658, 653-672, 665-684, 668-688, 670-706, 670-686, 670-685, 671-691, 671-687, 671-686, 672-688, 673-688, 679-703, 681-696, 682-697, 686-701, 686-706, 687-702, 687-703, 688-703, 689-708, 693-712, 695-714, 696-715, 697-716, 727-746, 739-754, 742-761, 748-767, 751-770, 754-773, 757-776, 760-779, 763-782, 766-785, 790-809, 793-812, 796-815, 811-830, 814-833, 817-836, 820-839, 822-844, 845-864, 854-873, 857-876, 863-882, 866-885, 872-891, 875-894, 878-897, 881-900, 884-903, 887-906, 899-918, 902-921, 905-924, 908-927, 911-930, 914-933, 936-955, 939-958, 951-970, 954-973, 957-976, 960-979, 963-982, 966-985, 969-988, 972-991, 975-994, 978-997, 996-1015, 1002-1021, 1025-1044, 1031-1050, 1034-1053, 1037-1056, 1046-1065, 1049-1068, 1052-1071, 1055-1074, 1058-1077, 1061-1080, 1064-1083, 1070-1089, 1073-1092, 1076-1095, 1082-1101, 1088-1107, 1094-1113, 1097-1116, 1100-1119, 1103-1122, 1106-1125, 1109-1128, 1112-1131, 1115-1134, 1121-1140, 1127-1146, 1153-1172,

1156-1175, 1159-1178, 1162-1181, 1165-1184, 1168-1191, 1174-1193, 1206-1225, 1209-1228, 1212-1231, 1215-1234, 1218-1237, 1221-1240, 1224-1243, 1227-1246, 1230-1249, 1233-1252, 1236-1255, 1239-1258, 1242-1261, 1245-1264, 1251-1270, 1254-1273, 1254-1279, 1257-1283, 1257-1276, 1258-1277, 1260-1279, 1261-1285, 1261-1276, 1261-1277, 1261-1280, 1262-1277, 1262-1278, 1263-1278, 1263-1279, 1263-1282, 1264-1279, 1264-1280, 1264-1283, 1265-1281, 1265-1284, 1266-1281, 1266-1282, 1266-1285, 1267-1282, 1267-1283, 1268-1283, 1268-1284, 1268-1287, 1269-1284, 1269-1285, 1270-1285, 1272-1291, 1275-1294, 1282-1303, 1286-1306, 1290-1309, 1293-1312, 1296-1315, 1299-1318, 1305-1324, 1311-1330, 1314-1333, 1317-1336, 1353-1381, 1356-1375, 1359-1378, 1498-1517, 1501-1520, 1504-1523, 1510-1529, 1553-1572, 1556-1575, 1559-1578, 1562-1581, 1565-1584, 1571-1590, 1574-1599, 1577-1606, 1577-1592, 1577-1593, 1577-1596, 1578-1593, 1578-1594, 1578-1597, 1579-1594, 1579-1595, 1579-1598, 1580-1595, 1580-1596, 1581-1596, 1581-1597, 1581-1600, 1582-1597, 1582-1598, 1582-1601, 1582-1602, 1583-1598, 1583-1599, 1583-1602, 1584-1599, 1584-1600, 1584-1603, 1585-1600, 1585-1601, 1585-1604, 1586-1601, 1586-1605, 1586-1602, 1587-1602, 1587-1603, 1587-1606, 1588-1603, 1588-1604, 1589-1604, 1589-1605, 1590-1605, 1590-1606, 1591-1606, 1604-1623, 1607-1626, 1630-1649, 1633-1652, 1645-1664, 1651-1670, 1654-1674, 1657-1676, 1660-1679, 1663-1682, 1666-1685, 1689-1708, 1695-1714, 1698-1717, 1701-1720, 1716-1735, 1778-1797, 1778-1794, 1778-1797, 1779-1795, 1779-1798, 1780-1795, 1780-1796, 1780-1799, 1781-1800, 1794-1813, 1895-1914, 1898-1917, 1901-1920, 1907-1926, 1910-1929, 1913-1932, 1916-1935, 1919-1938, 2278-2297, 2281-2300, and 2284-2303.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 80% inhibition: 13-32, 16-35, 19-38, 25-44, 28-47, 46-65, 49-68, 58-77, 59-80, 63-82, 98-120, 116-135, 137-159, 158-177, 167-186, 203-224, 205-224, 209-228, 218-237, 233-252, 236-263, 245-264, 253-272, 256-275, 257-276, 266-288, 281-300, 290-312, 293-312, 324-343, 339-358, 348-367, 358-378, 360-379, 361-383, 366-385, 373-392, 382-401, 405-424, 411-431, 411-426, 411-427, 411-430, 413-428, 414-433, 414-434, 415-430, 415-434, 416-431, 416-435, 417-436, 418-437, 422-441, 425-444, 434-453, 456-476, 458-473, 458-477, 464-483, 471-493, 488-507, 494-513, 512-531, 524-543, 527-546, 536-558, 560-579, 566-585, 572-591, 575-594, 584-603, 587-606, 608-627, 614-633, 617-636, 620-639, 623-642, 626-645, 629-648, 639-654, 641-656, 642-657, 643-658, 665-688, 670-687, 670-686, 671-686, 671-687, 671-691, 673-688, 679-699, 682-697, 682-706, 686-701, 687-702, 687-706, 687-703, 693-715, 727-746, 742-761, 748-767, 757-776, 766-785, 790-815, 814-833, 820-839, 822-844, 845-864, 854-873, 854-876, 863-885, 872-906, 878-897, 899-918, 905-933, 936-955, 951-979, 963-985, 966-985, 972-1015, 978-997, 1002-1021, 1025-1044, 1031-1056, 1049-1074, 1061-1083, 1070-1089, 1082-1101, 1088-11107, 1094-1119, 1109-1134, 1121-1140, 1127-1146, 1159-1187, 1171-1191, 1206-1228, 1209-1228, 1215-1255, 1215-1234, 1218-1237, 1221-1240, 1224-1243, 1227-1246, 1230-1249, 1233-1252, 1236-1255, 1245-1264, 1251-1279, 1251-1270, 1254-1273, 1254-1279, 1257-1276, 1258-1277, 1259-1278, 1260-1279, 1261-1285, 1261-1276, 1261-1277, 1261-1280, 1262-1277, 1262-1278, 1262-1281, 1263-1278, 1263-1282, 1264-1279, 1264-

1283, 1265-1284, 1266-1285, 1267-1282, 1267-1283, 1268-1283, 1268-1284, 1269-1284, 1269-1285, 1269-1288, 1270-1285, 1275-1294, 1282-1301, 1286-1306, 1293-1318, 1311-1333, 1326-1345, 1359-1378, 1553-1578, 1565-1584, 1571-1590, 1574-1599, 1577-1592, 1577-1596, 1577-1593, 1577-1596, 1578-1593, 1578-1594, 1578-1597, 1579-1595, 1579-1598, 1580-1596, 1580-1599, 1581-1596, 1581-1597, 1581-1600, 1582-1597, 1582-1601, 1582-1602, 1583-1598, 1583-1599, 1583-1602, 1584-1599, 1584-1603, 1585-1601, 1585-1604, 1586-1605, 1587-1602, 1587-1606, 1588-1603, 1589-1604, 1589-1605, 1657-1679, 1780-1795, 1780-1796, 1780-1799, 1913-1935, 2278-2297, 2281-2300, and 2284-2303.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 85% inhibition: 13-32, 16-35, 19-38, 25-44, 46-65, 59-80, 101-120, 140-159, 158-177, 167-186, 200-219, 205-224, 209-228, 233-252, 242-263, 253-272, 266-285, 281-300, 290-311, 293-312, 359-379, 361-381, 370-389, 382-401, 411-426, 411-430, 411-427, 413-428, 414-433, 415-430, 416-435, 417-436, 422-441, 456-476, 458-473, 470-493, 512-531, 524-543, 536-558, 566-585, 575-594, 587-606, 608-627, 614-636, 623-645, 639-654, 665-687, 671-686, 671-687, 680-699, 682-703, 687-706, 687-703, 727-746, 742-761, 757-776, 793-812, 822-843, 854-876, 854-873, 863-885, 878-900, 878-897, 887-906, 899-918, 905-927, 914-933, 936-955, 951-985, 966-985, 972-1015, 978-997, 1002-1021, 1025-1044, 1037-1056, 1049-1074, 1064-1083, 1070-1089, 1088-1107, 1094-1119, 1109-1128, 1121-1140, 1156-1175, 1162-1187, 1172-1191, 1206-1228, 1209-1228, 1215-1255, 1215-1234, 1218-1237, 1221-1240, 1224-1243, 1227-1246, 1230-1249, 1233-1252, 1236-1255, 1245-1264, 1251-1279, 1251-1270, 1254-1273, 1254-1279, 1257-1276, 1258-1277, 1259-1278, 1260-1279, 1261-1285, 1261-1276, 1261-1277, 1261-1280, 1262-1277, 1262-1278, 1262-1281, 1263-1278, 1263-1282, 1264-1279, 1264-1283, 1265-1284, 1266-1285, 1267-1282, 1267-1283, 1268-1284, 1269-1284, 1269-1285, 1269-1288, 1270-1285, 1275-1294, 1282-1301, 1293-1315, 1311-1330, 1359-1378, 1574-1593, 1577-1592, 1577-1593, 1577-1596, 1577-1606, 1578-1593, 1578-1594, 1578-1597, 1579-1598, 1580-1596, 1580-1599, 1581-1597, 1581-1600, 1582-1601, 1583-1598, 1583-1602, 1584-1603, 1585-1601, 1585-1604, 1586-1605, 1587-1602, 1588-1603, 1780-1799, 1780-1796, and 2278-2297, 2281-2300, and 2284-2303.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 90% inhibition: 13-32, 16-35, 60-80, 140-159, 158-177, 167-186, 242-261, 292-311, 362-381, 370-389, 382-401, 411-427, 411-426, 413-428, 415-430, 416-435, 422-441, 473-492, 617-636, 623-642, 639-654, 668-687, 680-699, 682-701, 684-703, 687-706, 727-746, 757-776, 824-843, 854-873, 854-876, 863-882, 878-897, 878-900, 887-906, 899-918, 905-927, 914-933, 936-955, 951-970, 960-985, 966-985, 972-1015, 978-997, 1025-1044, 1037-1056, 1070-1089, 1097-1119, 1109-1128, 1121-1140, 1165-1187, 1172-1191, 1206-1228, 1209-1228, 1215-1234, 1215-1234, 1215-1255, 1218-1237, 1221-1240, 1224-1243, 1227-1246, 1230-1249, 1233-1252, 1236-1255, 1245-1264, 1251-1279, 1251-1270, 1254-1273, 1254-1279, 1257-1276, 1258-1277, 1259-1278, 1260-1279, 1261-1285, 1261-1280, 1262-1278,

1261-1276, 1262-1281, 1262-1277, 1263-1282, 1263-1278, 1264-1283, 1265-1284, 1266-1285, 1268-1284, 1269-1284, 1269-1285, 1269-1288, 1296-1315, 1577-1605, 1577-1596, 1577-1593, 1577-1592, 1578-1597, 1581-1600, 1582-1601, 1583-1602, 1583-1598, 1585-1601, 1585-1604, 1586-1605, 1588-1603, 1780-1799, 1780-1796, 2278-2297, 2281-2300, and 2284-2303.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 95% inhibition: 411-426, 411-427, 413-428, 617-636, 623-642, 668-687, 680-699, 682-701, 854-873, 878-897, 887-906, 914-933, 966-985, 978-997, 1209-1228, 1215-1234, 1218-1237, 1221-1240, 1224-1243, 1227-1246, 1230-1249, 1233-1252, 1236-1255, 1245-1264, 1251-1270, 1254-1273, 1254-1279, 1257-1276, 1258-1277, 1259-1278, 1260-1279, 1261-1285, 1261-1280, 1262-1281, 1263-1282, 1263-1278, 1264-1283, 1265-1284, 1266-1285, 1268-1284, 1269-1288, 1577-1592, 1577-1596, 1577-1601, 1583-1598, 1585-1601, 1588-1603, 1780-1799, 2278-2297, 2281-2300, and 2284-2303.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a HBV nucleic acid and effect at least a 50% inhibition of a HBV mRNA, ISIS IDs: 510088, 510089, 510090, 510092, 510096, 510097, 510098, 510099, 510100, 510101, 510102, 505330, 509928, 510104, 509929, 510105, 509930, 510106, 510107, 510108, 510111, 510115, 509931, 510116, 510117, 510118, 510119, 510120, 510121, 509932, 510122, 509933, 510123, 509934, 510124, 509935, 510125, 510126, 510127, 510128, 510140, 146779, 505314, 505315, 505316, 505317, 146821, 505318, 509922, 505319, 509925, 505320, 509952, 505321, 505322, 505323, 505324, 505325, 505326, 505327, 505328, 505329, 509956, 509957, 509927, 509958, 510038, 505330, 509959, 510039, 509960, 510040, 509961, 510041, 509962, 509963, 505331, 505332, 509968, 509969, 510050, 510052, 505333, 505334, 505335, 505336, 509972, 146823, 509974, 505338, 505339, 509975, 505340, 509978, 505341, 509979, 510058, 505342, 509981, 510061, 505344, 505345, 509983, 505346, 509984, 505347, 505348, 505350, 505352, 505353, 505354, 505355, 505356, 146786, 505357, 505358, 505359, 505360, 509985, 509986, 509987, 509988, 505363, 505364, 505365, 505366, 146787, 510079, 524410, 524411, 524413, 524414, 524415, 524416, 524417, 524418, 524419, 524420, 524421, 524422, 524424, 524425, 524426, 524427, 524428, 524429, 524431, 524432, 524433, 524434, 524435, 524436, 524439, 524440, 524442, 524444, 524446, 524447, 524448, 524450, 524451, 524452, 524453, 524454, 524455, 524456, 524457, 524458, 524459, 524460, 524461, 524462, 524464, 524466, 524467, 524468, 524469, 524470, 524471, 524472, 524473, 524474, 524475, 524477, 524478, 524479, 524480, 524481, 524482, 524483, 524484, 524485, 524486, 524487, 524489, 524490, 524491, 524492, 524493, 524494, 524495, 524496, 524498, 524499, 524500, 524501, 524502, 524503, 524504, 524506, 524507, 524508, 524509, 524510, 524511, 524512, 524513, 524514, 524515, 524516, 524517, 524518, 524519, 524520, 524521, 524522, 524523, 524524, 524525, 524526, 524527, 524528, 524529, 524530, 524531, 524532, 524533, 524534, 524535, 524536, 524537, 524538, 524539,

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In certain embodiments, the following antisense compounds or oligonucleotides target a region of a HBV nucleic acid and effect at least a 50% inhibition of a HBV mRNA, SEQ ID NOs: 5, 6, 7, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 33, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 74, 83, 85, 86, 87, 88, 89, 92, 96, 98, 99, 100, 102, 103, 104, 106, 108, 109, 111, 112, 115, 117, 121, 122, 123, 124, 125, 126, 127, 128, 136, 137, 139, 140, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 153, 155, 157, 159, 161, 165, 166, 167, 168, 169, 171, 172, 173, 174, 175, 176, 177, 178,

179, 180, 181, 186, 187, 188, 189, 190, 191, 192, 193, 194, 197, 198, 199, 201, 203, 206, 207, 208, 209, 210, 211, 212, 213, 215, 217, 218, 220, 221, 222, 224, 225, 226, 227, 228, 230, 231, 232, 233, 234, 235, 236, 237, 240, 241, 242, 243, 244, 250, 283, 321, 322, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 335, 336, 337, 338, 339, 340, 342, 343, 344, 345, 346, 347, 350, 351, 353, 355, 357, 358, 359, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 399, 400, 401, 402, 403, 404, 405, 406, 408, 409, 410, 411, 412, 413, 414, 416, 417, 418, 419, 420 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 453, 454, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 473, 474, 475, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 539, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 564, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 582, 583, 585, 586, 588, 589, 590, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 622, 623, 624, 625, 626, 627, 628, 629, 631, 632, 633, 634, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 754, 755, 756, 757, 759, 760, 768, 769, 773, 777, 778, 779, 780, 781, 782, 783, 784, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 876, 877, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 903, 904, 905, 906, 909, 910, 933, 934, 949, 951, 955, 962, 964, 998, 1002, 1013, 1052, 1267, 1271, 1272, 1288, 1289, 1290, 1291, 1292, 1293, 1294, 1295, 1296, 1297, 1298, 1299, 1300, 1301, 1302, 1303, 1304, 1305, 1306, 1307, 1308, 1309, 1310, 1311, 1312, 1313, 1314, 1315, 1316, 1317, 1318, 1319, 1320, 1321, 1322, 1323, 1324, 1325, 1326, 1327, 1328, 1329, 1330, 1331, 1332, 1333, 1334, 1335, 1336, 1337, 1338, 1339, 1340, 1341, 1342, 1343, 1344, 1345, 1346, 1347, 1348, 1349, 1350, 1364, 1365, 1366, 1367, 1368, 1369, 1370, 1371, 1372, 1375, and 1376.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a HBV nucleic acid and effect at least a 60% inhibition of a HBV mRNA, ISIS IDs: 510090, 510100, 510102, 505330, 509928, 510104, 509929, 510105, 509930, 510106, 510107, 510111, 509931, 510116, 510117, 510118, 510119, 510120, 510121, 509932, 510122, 509933, 510123, 509934, 510124, 509935, 510125,

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In certain embodiments, the following antisense compounds or oligonucleotides target a region of a HBV nucleic acid and effect at least a 60% inhibition of a HBV mRNA, SEQ ID NOs: 7, 9, 10, 12, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 33, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 56, 83, 85, 86, 87, 88, 89, 92, 96, 98, 100, 102, 103, 112, 115, 117, 122, 123, 124, 125, 126, 127, 128, 136, 137, 139, 140, 142, 143, 145, 147, 149, 150, 151, 153, 155, 157, 159, 161, 166, 167, 168, 172, 174, 176, 177, 178, 179, 180, 181, 186, 187, 188, 189, 190, 191, 192, 193, 194, 198, 199, 201, 206, 207, 208, 209, 210, 211, 212, 213, 218, 220, 222, 224, 225, 226, 227, 228, 230, 231, 232, 233, 234, 240, 243, 321, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 335, 336, 337, 339, 342, 343, 344, 345, 346, 350, 351, 357, 358, 359, 362, 363, 364, 365, 366, 367, 368, 370, 371, 372, 375, 376, 377, 378, 379, 381, 382, 383, 384, 387, 388, 389, 390, 391, 392, 395, 396, 397, 399, 400, 401, 402, 403, 404, 405, 406, 409, 410, 411, 412, 413, 414, 416, 417, 418, 419, 420 421, 422, 423, 424, 425, 426, 427, 429, 430, 431, 433, 435, 436, 437, 438, 439, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 453, 456, 457, 459, 460, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 473, 474, 475, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 495, 496, 497, 498, 499, 500, 501, 503, 504, 505, 508, 509, 510, 512, 513, 514, 515, 516, 517, 520, 521, 524, 525, 526, 527, 528, 529, 530, 531, 532,

533, 535, 537, 539, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 564, 566, 567, 568, 569, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 583, 585, 586, 588, 589, 590, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 623, 624, 625, 626, 627, 628, 629, 631, 632, 634, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 652, 653, 654, 655, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 740, 741, 742, 744, 745, 754, 755, 756, 757, 759, 768, 777, 778, 779, 780, 781, 782, 783, 784, 787, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 833, 834, 835, 836, 837, 838, 840, 841, 842, 843, 844, 845, 848, 849, 850, 851, 852, 853, 854, 856, 858, 859, 860, 861, 862, 864, 865, 866, 867, 873, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 903, 904, 905, 906, 949, 964, 1271, 1288, 1289, 1290, 1291, 1292, 1293, 1294, 1295, 1296, 1297, 1298, 1299, 1300, 1301, 1302, 1303, 1304, 1305, 1306, 1307, 1308, 1309, 1310, 1311, 1312, 1313, 1314, 1315, 1316, 1317, 1318, 1319, 1320, 1321, 1322, 1323, 1324, 1325, 1326, 1327, 1328, 1329, 1330, 1331, 1332, 1333, 1334, 1335, 1336, 1337, 1338, 1339, 1340, 1341, 1342, 1343, 1344, 1345, 1346, 1347, 1348, 1349, 1350, 1365, 1366, 1367, 1368, 1369, 1370, 1371, 1372, and 1376.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a HBV nucleic acid and effect at least a 70% inhibition of a HBV mRNA, ISIS IDs: 510100, 505330, 509928, 509929, 509930, 510106, 509931, 510116, 510119, 510120, 510121, 509932, 510122, 509933, 510123, 509934, 510124, 509935, 146779, 505317, 146821, 505318, 505319, 505323, 505325, 505326, 505327, 509957, 505330, 505332, 505335, 509974, 505338, 505339, 509975, 505342, 509981, 505345, 505346, 505347, 505348, 146786, 505357, 505358, 505359, 505363, 524410, 524413, 524414, 524415, 524416, 524418, 524419, 524420, 524421, 524424, 524425, 524426, 524428, 524431, 524432, 524433, 524434, 524435, 524446, 524447, 524448, 524452, 524453, 524457, 524459, 524460, 524461, 524464, 524466, 524467, 524468, 524469, 524472, 524473, 524474, 524475, 524477, 524478, 524479, 524480, 524481, 524482, 524485, 524487, 524490, 524491, 524492, 524493, 524494, 524495, 524499, 524500, 524502, 524503, 524507, 524508, 524510, 524511, 524512, 524513, 524514, 524515, 524516, 524517, 524520, 524525, 524526, 524528, 524532, 524533, 524534, 524535, 524536, 524537, 524538, 524539, 524540, 524541, 524547, 524549, 524552, 524553, 524554, 524555, 524556, 524557, 524558, 524559, 524560, 524561, 524563, 524564, 524565, 524568, 524569, 524570, 524571, 524572, 524573, 524574, 524575, 524577, 524578, 524579, 524580, 524582, 524586, 524587, 524590, 524591, 524594, 524595, 524598, 524600, 524602, 524603, 524604, 524605, 524606, 524607, 524610, 524611, 524614, 524615, 524616, 524617, 524618, 524619, 524620, 524621, 524629, 524633, 524634, 524635, 524636, 524637, 524638,

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In certain embodiments, the following antisense compounds or oligonucleotides target a region of a HBV nucleic acid and effect at least a 70% inhibition of a HBV mRNA, SEQ ID NOs: 12, 17, 18, 20, 21, 22, 24, 25, 26, 27, 28, 29, 39, 40, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 83, 89, 92, 96, 98, 100, 103, 112, 123, 125, 126, 127, 136, 137, 139, 140, 142, 143, 145, 147, 149, 151, 153, 166, 167, 168, 174, 176, 177, 178, 179, 181, 186, 187, 188, 190, 198, 201, 207, 209, 210, 211, 212, 213, 224, 225, 226, 227, 232, 234, 240, 321, 324, 325, 326, 327, 329, 330, 331, 332, 335, 336, 337, 339, 342, 343, 344, 345, 346, 357, 358, 359, 363, 364, 368, 370, 371, 372, 375, 376, 377, 378, 379, 382, 383, 384, 387, 388, 389, 390, 391, 392, 395, 397, 400, 401, 402, 403, 404, 405, 409, 410, 412, 413, 417, 418, 420 421, 422, 423, 424, 425, 426, 427, 430, 435, 436, 438, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 457, 459, 462, 463, 464, 465, 466, 467, 468, 469, 470, 473, 474, 475, 478, 479, 480, 481, 482, 483, 484, 485, 487, 488, 489, 490, 492, 496, 497, 500, 501, 504, 505, 508, 510, 512, 513, 514, 515, 516, 517, 520, 521, 524, 525, 526, 527, 528, 529, 530, 531, 539, 543, 544, 545, 546, 547, 548, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 566, 567, 569, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 588, 589, 590, 595, 596, 597, 598, 599, 600, 601, 602, 605, 606, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 623, 624, 625, 626, 627, 628, 631, 632, 634, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 652, 653, 654, 657, 658, 659, 660, 661, 662, 663, 665, 666, 667, 669, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 682, 684, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 733, 734, 735, 736, 737, 738, 740, 741, 742, 745, 754, 755, 756, 757, 768, 777, 778, 779, 780, 781, 782, 784, 790, 791, 792, 793, 794, 795, 796, 797, 798,

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In certain embodiments, the following antisense compounds or oligonucleotides target a region of a HBV nucleic acid and effect at least a 80% inhibition of a HBV mRNA, ISIS IDs: 510100, 509931, 510116, 505317, 505319, 505323, 505326, 505327, 505330, 505339, 505346, 505347, 505358, 509934, 146786, 524414, 524415, 524416, 524418, 524419, 524425, 524426, 524431, 524432, 524434, 524446, 524447, 524452, 524459, 524460, 524466, 524469, 524475, 524477, 524478, 524479, 524482, 524485, 524490, 524491, 524492, 524493, 524494, 524495, 524499, 524502, 524503, 524507, 524510, 524511, 524512, 524520, 524525, 524528, 524532, 524533, 524534, 524535, 524536, 524540, 524541, 524547, 524552, 524553, 524556, 524561, 524564, 524565, 524568, 524570, 524571, 524572, 524573, 524578, 524580, 524586, 524590, 524591, 524594, 524595, 524602, 524604, 524606, 524607, 524610, 524611, 524614, 524616, 524617, 524618, 524619, 524620, 524621, 524633, 524634, 524635, 524636, 524637, 524641, 524643, 524644, 524646, 524649, 524650, 524651, 524657, 524662, 524664, 524667, 524670, 524678, 524679, 524680, 524686, 524688, 524690, 524691, 524692, 524695, 524698, 524699, 524701, 524702, 524704, 524705, 524706, 524707, 524708, 524709, 524713, 524715, 524716, 524717, 524718, 524721, 524726, 524727, 524728, 524729, 524730, 524731, 524733, 524734, 524735, 524737, 524739, 524741, 524742, 524743, 524747, 524748, 524749, 524751, 524752, 524754, 524758, 524760, 524762, 524763, 524764, 524767, 524768, 524769, 524771, 524773, 524777, 524778, 524779, 524780, 524781, 524783, 524784, 524788, 524789, 524791, 524792, 524793, 524794, 524795, 524796, 524797, 524798, 524801, 524803, 524804, 524805, 524806, 524807, 524808, 524809, 524810, 524811, 524813, 524816, 524819, 524822, 524823, 524824, 524827, 524828, 524829, 524833, 524842, 524844, 524880, 524881, 524882, 524884, 524886, 524887, 524888, 524889, 524890, 524891, 524893, 524907, 524908, 524980, 524986, 524987, 551921, 551924, 551925, 551953, 551956, 551957, 551984, 551986, 551987, 551989, 551990, 551993, 551994, 551995, 551996, 551997, 551998, 551999, 552000, 552005, 552006, 552018, 552019, 552020, 552021, 552022, 552023, 552024, 552025, 552026, 552027, 552028, 552029, 552030, 552031, 552032, 552033, 552034, 552039, 552044, 552046, 552050, 552051, 552052, 552053, 552054, 552055, 552056, 552057, 552058, 552059, 552060, 552061, 552062, 552063, 552064, 552065, 552073, 552077, 552078, 552079, 552080, 552082, 552083, 552084, 552085, 552086, 552087, 552088, 552089, 552090, 552091, 552092, 552093, 552094, 552095, 552096, 552097, 552098, 552138, 552139, 552145, 552146, 552147, 552149, 552192, 552193, 552199, 552200, 552201, 552207, 552246, 552247, 552253, 552301, 552307, 552308, 552310, 552317, 552347, 552348, 552354, 552355, 552360, 552361, 552362, 552363,

552364, 552365, 552366, 552367, 552371, 552375, 552464, 552465, 552521, 552808, 552816, 552817, 552818, 552819, 552820, 552822, 552824, 552834, 552844, 552849, 552850, 552851, 552852, 552853, 552854, 552916, 552922, 552923, 552925, 552930, 552931, 552932, 552933, 552936, 552937, 552938, 552939, 552942, 552943, 552944, 552980, 552988, 552989, 552996, 552998, 553002, 553003, 566828, 566829, 566830, 566831, 566832, 577120, 577121, 577122, 577123, 577124, 577125, 577126, 577127, 577128, 577130, 577131, 577132, 577133, 577134, 577135, 577136, and 582666.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a HBV nucleic acid and effect at least a 80% inhibition of a HBV mRNA, SEQ ID NOs: 17, 20, 22, 24, 26, 28, 39, 40, 50, 51, 83, 89, 103, 123, 126, 127, 136, 137, 143, 147, 149, 168, 176, 177, 178, 179, 187, 188, 210, 211, 212, 224, 225, 226, 227, 232, 325, 326, 327, 329, 330, 336, 337, 342, 343, 345, 357, 358, 363, 370, 371, 376, 379, 387, 388, 389, 392, 395, 400, 401, 402, 403, 404, 405, 409, 412, 413, 417, 420 421, 422, 430, 435, 438, 442, 443, 444, 445, 446, 450, 451, 457, 462, 463, 466, 474, 475, 478, 480, 481, 482, 483, 488, 490, 496, 500, 501, 504, 505, 512, 514, 516, 517, 520, 521, 524, 526, 527, 528, 529, 530, 531, 543, 544, 545, 546, 547, 551, 553, 554, 555, 559, 560, 561, 567, 572, 574, 577, 580, 588, 589, 590, 596, 598, 600, 601, 602, 605, 608, 609, 611, 612, 614, 615, 616, 617, 618, 619, 623, 625, 626, 627, 628, 631, 636, 637, 638, 639, 640, 641, 643, 644, 645, 646, 648, 650, 652, 653, 654, 658, 659, 660, 662, 663, 665, 669, 671, 673, 674, 675, 678, 679, 680, 682, 684, 688, 689, 690, 691, 692, 694, 695, 699, 700, 702, 703, 704, 705, 706, 707, 708, 709, 712, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 725, 728, 731, 734, 735, 736, 740, 741, 745, 756, 791, 792, 793, 795, 797, 798, 799, 800, 801, 802, 804, 805, 806, 807, 819, 820, 892, 898, 899, 1292, 1293, 1295, 1296, 1301, 1302, 1303, 1304, 1305, 1306, 1307, 1308, 1310, 1312, 1316, 1322, 1324, 1325, 1326, 1327, 1330, 1331, 1332, 1333, 1334, 1335, 1338, 1339, 1340, 1341, 1344, 1345, 1349, 1350, 1368, 1372, and 1376.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a HBV nucleic acid and effect at least a 90% inhibition of a HBV mRNA, ISIS IDs: 524414, 524415, 524432, 524460, 524466, 524469, 524475, 524477, 524493, 524512, 524535, 524540, 524552, 524561, 524572, 524617, 524619, 524634, 524641, 524644, 524657, 524667, 524691, 524698, 524699, 524701, 524706, 524707, 524709, 524713, 524715, 524716, 524718, 524721, 524726, 524729, 524730, 524731, 524733, 524734, 524735, 524739, 524743, 524754, 524763, 524764, 524767, 524771, 524780, 524781, 524784, 524788, 524789, 524791, 524792, 524793, 524794, 524795, 524796, 524797, 524798, 524801, 524803, 524804, 524805, 524806, 524807, 524808, 524809, 524810, 524811, 524822, 524827, 524842, 551986, 551987, 551989, 552005, 552018, 552019, 552020, 552021, 552022, 552023, 552025, 552046, 552050, 552051, 552052, 552053, 552054, 552055, 552057, 552082, 552083, 552084, 552085, 552086, 552087, 552088, 552089, 552092, 552093, 552096, 552097, 552307, 552317, 552355, 552361, 552362, 552363, 552817, 552851, 552922, 552923, 566828, 566829, 566830, 566831, 566832, 577120, 577121, 577122,

577123, 577124, 577125, 577126, 577127, 577128, 577130, 577131, 577132, 577134, 577135, 577136, and 582666.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a HBV nucleic acid and effect at least a 90% inhibition of a HBV mRNA, SEQ ID NOs: 17, 24, 50, 51, 137, 143, 147, 176, 211, 212, 224, 226, 227, 325, 326, 343, 371, 376, 379, 403, 422, 445, 450, 462, 482, 527, 529, 544, 551, 554, 567, 577, 601, 608, 609, 611, 616, 617, 619, 623, 625, 626, 628, 631, 636, 639, 640, 641, 643, 644, 645, 646, 650, 654, 665, 674, 675, 678, 682, 691, 692, 695, 699, 700, 702, 703, 704, 705, 706, 707, 708, 709, 712, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 735, 801, 804, 805, 807, 1296, 1302, 1303, 1304, 1312, 1325, 1326, 1332, 1334, 1340, 1345, 1349, and 1376.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a HBV nucleic acid and effect at least a 95% inhibition of a HBV mRNA, ISIS IDs: 524619, 524634, 524641, 505339, 524698, 524709, 524718, 524731, 524734, 524789, 524791, 524792, 524793, 524794, 524795, 524796, 524797, 524798, 524801, 524803, 524804, 524805, 524806, 505346, 146785, 524807, 505347, 524808, 524809, 524810, 524811, 146786, 525101, 525102, 525103, 525107, 525108, 525109, 525110, 525111, 525112, 525113, 525114, 525115, 525116, 525117, 525118, 525119, 525120, 552018, 552050, 552019, 552051, 552020, 552052, 551987, 552021, 552053, 552005, 552022, 552054, 551989, 552023, 552055, 552084, 552085, 552086, 552087, 552088, 552361, 552317, 566831, 577123, 577124, 566830, 566828, 566829, 577127, 577135, 577132, 577136, 566832, and 577122.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a HBV nucleic acid and effect at least a 95% inhibition of a HBV mRNA, SEQ ID NOs: 17, 50, 137, 143, 187, 210, 212, 224, 529, 544, 551, 608, 619, 628, 641, 645, 700, 702, 703, 704, 705, 706, 707, 708, 709, 712, 715, 716, 717, 718, 719, 720, 721, 722,723, 1014, 1015, 1016, 1020, 1021, 1022, 1023, 1024, 1025, 1026, 1027, 1028, 1029, 1030, 1031, 1032, 1033, 1236, 1302, 1312, 1334, 1340, 1345, 1349.

Certain embodiments provide methods of treating HBV related disease, disorder, or condition in an animal, comprising administering to an animal in need thereof a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 5-310, 321-802, 804-1272, 1288-1350 , 1364-1372, 1375, 1376, and 1379.

Certain embodiments provide methods of treating HBV related disease, disorder, or condition in an animal, comprising administering to an animal in need thereof a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 10 contiguous nucleobases of

any of the nucleobase sequences of SEQ ID NOs: 17, 51, 86, 93, 95, 98, 100, 102, 104, 106, 109, 112, 115, 117, 137, 140, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 167, 168, 176, 177- 179, 181, 188, 190- 192, 194, 199, 201, 208, 209, 211, 226, 230-237, 244, 245, 247, 252, 254, 256, 258, 260, 262, 264, 266, 271, 1318-1347, 1364- 1372, 1375, 1376, and 1379, wherein at least one nucleoside of the modified oligonucleotide comprises at least one 2'-O-methoxyethyl sugar and/or constrained ethyl (cEt) sugar. In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, or 4 sugar modified nucleosides.

Certain embodiments provide a method of reducing HBV expression in an animal comprising administering to the animal a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV and having a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 5-310, 321-802, 804-1272, 1288-1350 , 1364-1372, 1375, 1376, and 1379.

Certain embodiments provide a method of reducing HBV expression in an animal comprising administering to the animal a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV and having a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17, 51, 86, 93, 95, 98, 100, 102, 104, 106, 109, 112, 115, 117, 137, 140, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 167, 168, 176, 177- 179, 181, 188, 190- 192, 194, 199, 201, 208, 209, 211, 226, 230-237, 244, 245, 247, 252, 254, 256, 258, 260, 262, 264, 266, 271, 1318-1347, 1364- 1372, 1375, and1376, wherein at least one nucleoside of the modified oligonucleotide comprises at least one 2'-O-methoxyethyl sugar and/or constrained ethyl (cEt) sugar. In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 9 or 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, or 5 sugar modified nucleosides.

Certain embodiments provide a method of preventing, ameliorating or treating an HBV-related disease, disorder or condition in an animal comprising administering to the animal a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV and having a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17, 51, 86, 93, 95, 98, 100, 102, 104, 106, 109, 112, 115, 117, 137, 140, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 167, 168, 176, 177- 179, 181, 188, 190- 192, 194, 199, 201, 208, 209, 211, 226, 230-237, 244, 245, 247, 252, 254, 256, 258, 260, 262, 264, 266, 271, 1318-1347, 1364- 1372, 1375, and1376, wherein at least one

nucleoside of the modified oligonucleotide comprises at least one 2'-O-methoxyethyl sugar and/or constrained ethyl (cEt) sugar. In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 9 or 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, or 5 sugar modified nucleosides.

Certain embodiments provide methods of treating HBV related disease, disorder, or condition in an animal, comprising administering to an animal in need thereof a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 5, 15, 16, 33, 39-95, 123-135, 163-175, 180-310, 321-406, 413-455, 461-802, or 804-1272.

Certain embodiments provide methods of treating HBV related disease, disorder, or condition in an animal, comprising administering to an animal in need thereof a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 6-14, 17-32, 34-38, 96-122, 136-162, 176-179, 407-412, 456-462, 523-538 wherein at least one nucleoside of the modified oligonucleotide comprises at least one 2'-O-methoxyethyl sugar.

In certain embodiments, the modified oligonucleotide is 14 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-3 or 2 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 9 or 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 2-4 or 3 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, or 5 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 17 nucleosides in length and has a gap segment of 9 or 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 2-4 or 3-4 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, 5, or 6 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 18 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 3-5, or 4 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 20 nucleosides in length and

has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, or 5 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, 5, 6, 7, or 8 sugar modified nucleosides.

Certain embodiments provide a method of reducing HBV expression in an animal comprising administering to the animal a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV and having a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 5-310, 321-802, 804-1272, or 1288-1350.

Certain embodiments provide a method of reducing HBV expression in an animal comprising administering to the animal a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV and having a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 5, 15, 16, 33, 39-95, 123-135, 163-175, 180-310, 321-406, 413-455, 461-802, or 804-1272.

Certain embodiments provide a method of reducing HBV expression in an animal comprising administering to the animal a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV and having a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 6-14, 17-32, 34-38, 96-122, 136-162, 176-179, 407-412, 456-462, 523-538 wherein at least one nucleoside of the modified oligonucleotide comprises at least one 2'-O-methoxyethyl sugar.

In certain embodiments, the modified oligonucleotide is 14 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-3 or 2 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 9 or 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, or 5 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 2-4 or 3 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 17 nucleosides in length and has a gap segment of 9 or 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap

each independently having 2, 3, 4, 5, or 6 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 17 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 2-4 or 3-4 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 18 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 3-5, or 4 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 20 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, 5, 6, 7, or 8 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 20 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, or 5 sugar modified nucleosides.

Certain embodiments provide a method of preventing, ameliorating or treating an HBV-related disease, disorder or condition in an animal comprising administering to the animal a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV and having a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 5-310, 321-802, 804-1272, or 1288-1350, wherein at least one nucleoside of the modified oligonucleotide comprises at least one 2'-O-methoxyethyl sugar. In certain embodiments, the modified oligonucleotide has a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 5-310, 321-802, or 804-1272. In certain embodiments, the modified oligonucleotide has a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 5, 15, 16, 33, 39-95, 123-135, 163-175, 180-310, 321-406, 413-455, 461-802, or 804-1272. In certain embodiments, the modified oligonucleotide has a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 6-14, 17-32, 34-38, 96-122, 136-162, 176-179, 407-412, 456-462, 523-538 wherein at least one nucleoside of the modified oligonucleotide comprises at least one 2'-O-methoxyethyl sugar. In certain embodiments, the modified oligonucleotide is 14 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-3 or 2 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 9 or 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, or 5 sugar modified nucleosides. In certain embodiments, the modified

oligonucleotide is 16 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 2-4 or 3 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 17 nucleosides in length and has a gap segment of 9 or 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, 5, or 6 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 17 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 2-4 or 3-4 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 18 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 3-5, or 4 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 20 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 2, 3, 4, 5, 6, 7, or 8 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 20 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, or 5 sugar modified nucleosides.

Examples of HBV-related diseases, disorders or conditions include, but are not limited to chronic HBV infection, jaundice, liver cancer, liver inflammation, liver fibrosis, liver cirrhosis, liver failure, diffuse hepatocellular inflammatory disease, hemophagocytic syndrome, serum hepatitis, HBV viremia, and conditions having symptoms which may include any or all of the following: flu-like illness, weakness, aches, headache, fever, loss of appetite, diarrhea, nausea and vomiting, pain over the liver area of the body, clay- or grey-colored stool, itching all over, and dark-colored urine, when coupled with a positive test for presence of a hepatitis B virus, a hepatitis B viral antigen, or a positive test for the presence of an antibody specific for a hepatitis B viral antigen.

Certain embodiments provide a method of reducing HBV mRNA expression in an animal comprising administering to the animal a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV. In certain embodiments, reduction of HBV mRNA expression in an animal prevents, ameliorates or treats an HBV-related disease, disorder or condition. In certain embodiments, reduction of HBV mRNA expression in an animal prevents, ameliorates or treats liver disease. In certain embodiments, the HBV mRNA expression is reduced by at least 5%, 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%.

Certain embodiments provide a method of reducing HBV protein levels in an animal comprising administering to the animal a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV. In certain embodiments, reduction of HBV protein levels in an animal prevents, ameliorates or treats an HBV-related disease, disorder or condition. In certain embodiments, reduction of HBV protein levels in an animal prevents, ameliorates or treats liver disease. In certain embodiments, the HBV protein level is reduced by at least 5%, 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%.

Certain embodiments provide a method of reducing HBV DNA levels in an animal comprising administering to the animal a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV. In certain embodiments, reduction of HBV DNA levels in an animal prevents, ameliorates or treats an HBV-related disease, disorder or condition. In certain embodiments, the mammal may be human, and the hepatitis B virus may be a human hepatitis B virus. More particularly, the human hepatitis B virus may be any of the human geographical genotypes: A (Northwest Europe, North America, Central America); B (Indonesia, China, Vietnam); C (East Asia, Korea, China, Japan, Polynesia, Vietnam); D (Mediterranean area, Middle East, India); E (Africa); F (Native Americans, Polynesia); G (United States, France); or H (Central America). In certain embodiments, reduction of HBV DNA levels in an animal prevents, ameliorates or treats liver disease. In certain embodiments, the HBV DNA level is reduced by at least 5%, 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%.

Certain embodiments provide a method of reducing HBV antigen levels in an animal comprising administering to the animal a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV. In certain embodiments, the antigen is HBsAG or HBeAG. In certain embodiments, reduction of HBV antigen levels in an animal prevents, ameliorates or treats an HBV-related disease, disorder or condition. In certain embodiments, reduction of HBV antigen levels in an animal prevents, ameliorates or treats liver disease. In certain embodiments, the HBV antigen levels are reduced by at least 5%, 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%.

Certain embodiments provide a method of reducing HBV DNA and HBV antigen in a animal infected with a hepatitis B virus, comprising administering to the animal a compound or composition described herein. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV. In certain embodiments, the antigen is HBsAG or HBeAG. In certain embodiments, the amount of HBV antigen may be sufficiently reduced to result in seroconversion, defined as serum HBeAg absence plus serum HBeAb presence if monitoring

HBeAg as the determinant for seroconversion, or defined as serum HBsAg absence if monitoring HBsAg as the determinant for seroconversion, as determined by currently available detection limits of commercial ELISA systems.

Certain embodiments provide a method for treating an animal with a HBV related disease, disorder or condition comprising: a) identifying said animal with the HBV related disease, disorder or condition, and b) administering to said animal a therapeutically effective amount of a compound or composition comprising a modified oligonucleotide consisting of 14 to 20 linked nucleosides and having a nucleobase sequence at least 90% complementary to any of SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363, as measured over the entirety of said modified oligonucleotide. In certain embodiments, the therapeutically effective amount of the compound or composition administered to the animal treats or reduces the HBV related disease, disorder or condition, or a symptom thereof, in the animal. In certain embodiments, the HBV related disease, disorder or condition is a liver disease. In certain embodiments, the related disease, disorder or condition is chronic HBV infection, jaundice, liver cancer, liver inflammation, liver fibrosis, liver cirrhosis, liver failure, diffuse hepatocellular inflammatory disease, hemophagocytic syndrome, serum hepatitis, HBV viremia, or liver disease–related to transplantation.

Certain embodiments provide a method for treating an animal with a HBV related disease, disorder or condition comprising: a) identifying said animal with the HBV related disease, disorder or condition, and b) administering to said animal a therapeutically effective amount of a compound or composition comprising a modified oligonucleotide consisting of 14 to 20 linked nucleosides and having a nucleobase sequence at least 90% complementary to SEQ ID NO: 1, as measured over the entirety of said modified oligonucleotide. In certain embodiments, the therapeutically effective amount of the compound or composition administered to the animal treats or reduces the HBV related disease, disorder or condition, or a symptom thereof, in the animal. In certain embodiments, the HBV related disease, disorder or condition is a liver disease. In certain embodiments, the related disease, disorder or condition is chronic HBV infection, jaundice, liver cancer, liver inflammation, liver fibrosis, liver cirrhosis, liver failure, diffuse hepatocellular inflammatory disease, hemophagocytic syndrome, serum hepatitis, HBV viremia, or liver disease–related to transplantation.

In certain embodiments, HBV has the sequence as set forth in GenBank Accession Numbers U95551.1 (incorporated herein as SEQ ID NO: 1) or any variant or fragment thereof. In certain embodiments, HBV has truncated portions of the human sequence as set forth in SEQ ID NOs: 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363.

In certain embodiments, the animal is a human.

In certain embodiments, the compounds or compositions are designated as a first agent. In certain embodiments, the methods comprise administering a first agent and one or more second agents. In certain embodiments, the methods comprise administering a first agent and one or more second agents. In certain embodiments, the first agent and one or more second agents are co-administered. In certain embodiments the first agent and one or more second agents are co-administered sequentially or concomitantly.

In certain embodiments, the one or more second agents are also a compound or composition described herein. In certain embodiments, the one or more second agents are different from a compound or composition described herein. Examples of one or more second agents include, but are not limited to, an anti-inflammatory agent, chemotherapeutic agent or anti-infection agent.

In other related embodiments, the additional therapeutic agent may be an HBV agent, an HCV agent, a chemotherapeutic agent, an antibiotic, an analgesic, a non-steroidal anti-inflammatory (NSAID) agent, an antifungal agent, an antiparasitic agent, an anti-nausea agent, an anti-diarrheal agent, or an immunosuppressant agent.

In certain embodiments, the one or more second agents are an HBV agent. In certain embodiments the HBV agent can include, but is not limited to, interferon alpha-2b, interferon alpha-2a, and interferon alphacon-1 (pegylated and unpegylated), ribavirin; an HBV RNA replication inhibitor; a second antisense oligomer; an HBV therapeutic vaccine; an HBV prophylactic vaccine; lamivudine (3TC); entecavir (ETV); tenofovir diisoproxil fumarate (TDF); telbivudine (LdT); adefovir; or an HBV antibody therapy (monoclonal or polyclonal).

In certain embodiments, the one or more second agents are an HCV agent. In certain embodiments the HBV agent can include, but is not limited to interferon alpha-2b, interferon alpha-2a, and interferon alphacon-1 (pegylated and unpegylated); ribavirin; an HCV RNA replication inhibitor (e.g., ViroPharma's VP50406 series); an HCV antisense agent; an HCV therapeutic vaccine; an HCV protease inhibitor; an HCV helicase inhibitor; or an HCV monoclonal or polyclonal antibody therapy.

In certain embodiments, the one or more second agents are an anti-inflammatory agent (i.e., an inflammation lowering therapy). In certain embodiments the inflammation lowering therapy can include, but is not limited to, a therapeutic lifestyle change, a steroid, a NSAID or a DMARD. The steroid can be a corticosteroid. The NSAID can be an aspirin, acetaminophen, ibuprofen, naproxen, COX inhibitors, indomethacin and the like. The DMARD can be a TNF inhibitor, purine synthesis inhibitor, calcineurin inhibitor, pyrimidine synthesis inhibitor, a sulfasalazine, methotrexate and the like.

In certain embodiments, the one or more second agents are a chemotherapeutic agent (i.e., a cancer treating agent). Chemotherapeutic agents can include, but are not limited to, daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide, trimetrexate, teniposide, cisplatin, gemcitabine and diethylstilbestrol (DES).

In certain embodiments, the one or more second agents are an anti-infection agent. Examples of anti-infection agents include, but are not limited to, antibiotics, antifungal drugs and antiviral drugs.

In certain embodiments, administration comprises parenteral administration.

Certain embodiment provides a method for reducing an amount of HBV mRNA, DNA, protein and/or an amount of HBV antigen in a mammal infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a pharmaceutical composition as described above to a mammal in need thereof so as to reduce the hepatitis B virus infection and the hepatitis B antigen, compared to the amount of HBV mRNA, protein and an amount of HBV antigen in the mammal before treatment. In some embodiments, the mammal may be human, and the hepatitis B virus may be a human hepatitis B virus. More particularly, the human hepatitis B virus may be any of the human geographical genotypes: A (Northwest Europe, North America, Central America); B (Indonesia, China, Vietnam); C (East Asia, Korea, China, Japan, Polynesia, Vietnam); D (Mediterranean area, Middle East, India); E (Africa); F (Native Americans, Polynesia); G (United States, France); or H (Central America).

In certain embodiments, a method is provided for reducing an amount of HBV mRNA, DNA, protein and/or an amount of HBV antigen in a mammal infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a pharmaceutical composition as described above to a mammal in need thereof so as to reduce the hepatitis B virus infection and the hepatitis B antigen, compared to the amount of HBV mRNA, protein and an amount of HBV antigen in the mammal before treatment, wherein the amount of mRNA is reduced at least 70% compared to the amount before administration of the modified antisense oligonucleotide. In certain embodiments, a method is provided for reducing an amount of HBV mRNA, DNA, protein and/or an amount of HBV antigen in a mammal infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a pharmaceutical composition as

described above to a mammal in need thereof so as to reduce the hepatitis B virus infection and the hepatitis B antigen, compared to the amount of HBV mRNA, protein and an amount of HBV antigen in the mammal before treatment, wherein the amount of mRNA is reduced at least 75% compared to the amount before administration of the modified antisense oligonucleotide. In certain embodiments, a method is provided for reducing an amount of HBV mRNA, DNA, protein and/or an amount of HBV antigen in a mammal infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a pharmaceutical composition as described above to a mammal in need thereof so as to reduce the hepatitis B virus infection and the hepatitis B antigen, compared to the amount of HBV mRNA, protein and an amount of HBV antigen in the mammal before treatment, wherein the amount of mRNA is reduced at least 80% compared to the amount before administration of the modified antisense oligonucleotide. In certain embodiments, a method is provided for reducing an amount of HBV mRNA, DNA, protein and/or an amount of HBV antigen in a mammal infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a pharmaceutical composition as described above to a mammal in need thereof so as to reduce the hepatitis B virus infection and the hepatitis B antigen, compared to the amount of HBV mRNA, protein and an amount of HBV antigen in the mammal before treatment, wherein the amount of mRNA is reduced at least 85% compared to the amount before administration of the modified antisense oligonucleotide. In certain embodiments, a method is provided for reducing an amount of HBV mRNA, DNA, protein and/or an amount of HBV antigen in a mammal infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a pharmaceutical composition as described above to a mammal in need thereof so as to reduce the hepatitis B virus infection and the hepatitis B antigen, compared to the amount of HBV mRNA, protein and an amount of HBV antigen in the mammal before treatment, wherein the amount of mRNA is reduced at least 90% compared to the amount before administration of the modified antisense oligonucleotide. In certain embodiments, a method is provided for reducing an amount of HBV mRNA, DNA, protein and/or an amount of HBV antigen in a mammal infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a pharmaceutical composition as described above to a mammal in need thereof so as to reduce the hepatitis B virus infection and the hepatitis B antigen, compared to the amount of HBV mRNA, protein and an amount of HBV antigen in the mammal before treatment, wherein the amount of mRNA is reduced at least 95% compared to the amount before administration of the modified antisense oligonucleotide. In related methods, the HBV antigen may be HBsAg or may be HBeAg, and more particularly, the amount of HBV antigen may be sufficiently reduced to result in seroconversion, defined as serum HBeAg absence plus serum HBeAb presence if monitoring HBeAg as the determinant for seroconversion, or defined as serum HBsAg absence if monitoring HBsAg as the determinant for seroconversion, as determined by currently available detection limits of commercial ELISA systems.

Certain embodiment provides a method for promoting seroconversion of a hepatitis B virus in a

mammal infected with HBV, the method comprising administering a therapeutically effective amount of a pharmaceutical composition as described above to a mammal infected with hepatitis B; monitoring for presence of HBeAg plus HBeAb in a serum sample of the mammal, or monitoring for presence of HBsAg in a serum sample of the mammal, such that the absence of HBeAg plus the presence of HBeAb in the serum sample if monitoring HBeAg as the determinant for seroconversion, or the absence of HBsAg in the serum sample if monitoring HBsAg as the determinant for seroconversion, as determined by current detection limits of commercial ELISA systems, is indication of seroconversion in the mammal.

Certain embodiments provide the use of a compound or composition as described herein for preventing, ameliorating or treating liver disease, or symptom thereof, in an animal. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to HBV. In certain embodiments, the modified oligonucleotide has a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 17, 51, 86, 93, 95, 98, 100, 102, 104, 106, 109, 112, 115, 117, 137, 140, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 167, 168, 176, 177- 179, 181, 188, 190- 192, 194, 199, 201, 208, 209, 211, 226, 230-237, 244, 245, 247, 252, 254, 256, 258, 260, 262, 264, 266, 271, 1318-1347, 1364- 1372, 1375, 1376, and 1379.

In certain embodiments, the compounds or compositions as described herein are efficacious by virtue of having at least one of an *in vitro* IC_{50} of less than 250 nM, less than 200 nM, less than 150 nM, less than 100 nM, less than 90 nM, less than 80 nM, less than 70 nM, less than 65 nM, less than 60 nM, less than 55 nM, less than 50 nM, less than 49 nM, less than 47 nM, less than 46 nM, when delivered to HepG2.2.1 cells. In certain embodiments inhibition is measured with primer probe set RTS3370, as described herein.

In certain embodiments, the compounds or compositions as described herein are efficacious by virtue of having at least one of an *in vitro* IC_{50} of less than 250 nM, less than 200 nM, less than 100 nM, less than 90 nM, less than 80 nM, less than 70 nM, less than 60 nM, less than 50 nM, less than 40 nM, less than 35 nM, less than 34 nM, less than 33 nM, less than 32 nM, less than 31 nM, when delivered to HepG2.2.1 cells. In certain embodiments inhibition is measured with primer probe set RTS3371, as described herein.

In certain embodiments, the compounds or compositions as described herein are efficacious by virtue of having at least one of an *in vitro* IC_{50} of less than 20 μM, less than 10 μM, less than 9.5 μM, less than 9.0 μM, less than 8.5 μM, less than 8.0 μM, less than 7.5 μM, less than 7.0 μM, less than 6.5 μM, less than 6.0 μM, less than 5.5 μM, less than 5.0 μM, less than 4.5 μM, less than 4.0 μM, less than 3.5 μM, less than 3.0 μM, less than 2.5 μM, when delivered to HepG2.2.1 cells as described herein.

In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having at least one of an increase an ALT or AST value of no more than 4 fold, 3 fold, or 2

fold over saline treated animals or an increase in liver, spleen, or kidney weight of no more than 30%, 20%, 15%, 12%, 10%, 5%, or 2%. In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having no increase of ALT or AST over saline treated animals. In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having no increase in liver, spleen, or kidney weight over saline treated animals. In certain embodiments, these compounds or compositions include ISIS 146779, ISIS 146786, ISIS 505317, ISIS 505329, ISIS 505332, ISIS 505346, ISIS 505347, ISIS 505358, ISIS 509926, ISIS 509927, ISIS 509932, ISIS 509934, ISIS 509960, ISIS 509974, ISIS 510038, ISIS 510039, ISIS 510040, ISIS 510041, ISIS 510050ISIS 509975, ISIS 510100, ISIS 510106, and ISIS 510116. In certain embodiments, such compounds or compositions include compounds comprising the nucleobase sequence of any one of SEQ ID NOs: 5-310, 321-802, or 804-1272. In certain embodiments, such compounds or compositions include compounds comprising the nucleobase sequence of any one of SEQ ID NOs: 5, 15, 16, 33, 39-95, 123-135, 163-175, 180-310, 321-406, 413-455, 461-802, or 804-1272. In certain embodiments, such compounds or compositions include compounds comprising the nucleobase sequence of any one of SEQ ID NOs: 6-14, 17-32, 34-38, 96-122, 136-162, 176-179, 407-412, 456-462, 523-538 (update SEQ ID NOs) wherein at least one nucleoside of the modified oligonucleotide comprises at least one 2'-O-methoxyethyl sugar. In certain embodiments, the modified oligonucleotide is 14 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-3 or 2 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 16 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 2-4 or 3 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 17 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 2-4 or 3-4 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 18 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, 3-5, or 4 sugar modified nucleosides. In certain embodiments, the modified oligonucleotide is 20 nucleosides in length and has a gap segment of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide has a wing segment on the 5' end and 3' end of the gap each independently having 1-5, or 5 sugar modified nucleosides.

Certain embodiments provide the use of a compound or composition as described herein in the manufacture of a medicament for treating, ameliorating, delaying or preventing an HBV-related disease, disorder or condition in an animal.

Certain embodiments provide the use of a compound or composition as described herein in the manufacture of a medicament for treating, ameliorating, delaying or preventing liver disease in an animal.

Certain embodiments provide a kit for treating, preventing, or ameliorating an HBV-related disease, disorder or condition, or a symptom thereof, as described herein wherein the kit comprises: a) a compound or compositions as described herein; and optionally b) an additional agent or therapy as described herein. The kit can further include instructions or a label for using the kit to treat, prevent, or ameliorate the HBV-related disease, disorder or condition.

Antisense compounds

Oligomeric compounds include, but are not limited to, oligonucleotides, oligonucleosides, oligonucleotide analogs, oligonucleotide mimetics, antisense compounds, antisense oligonucleotides, and siRNAs. An oligomeric compound may be "antisense" to a target nucleic acid, meaning that is is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

In certain embodiments, an antisense compound has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted. In certain such embodiments, an antisense oligonucleotide has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted.

In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 10-30 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 12 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 12 to 22 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 14 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 14 to 20 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 15 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 15 to 20 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 16 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 16 to 20 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 17 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 17 to 20 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 18 to 30 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 18 to 21 subunits in length. In certain embodiments, an antisense compound targeted to a HBV

nucleic acid is 18 to 20 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 20 to 30 subunits in length. In other words, such antisense compounds are from 12 to 30 linked subunits, 14 to 30 linked subunits, 14 to 20 subunits, 15 to 30 subunits, 15 to 20 subunits, 16 to 30 subunits, 16 to 20 subunits, 17 to 30 subunits, 17 to 20 subunits, 18 to 30 subunits, 18 to 20 subunits, 18 to 21 subunits, 20 to 30 subunits, or 12 to 22 linked subunits, respectively. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 14 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 16 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 17 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 18 subunits in length. In certain embodiments, an antisense compound targeted to a HBV nucleic acid is 20 subunits in length. In other embodiments, the antisense compound is 8 to 80, 12 to 50, 13 to 30, 13 to 50, 14 to 30, 14 to 50, 15 to 30, 15 to 50, 16 to 30, 16 to 50, 17 to 30, 17 to 50, 18 to 22, 18 to 24, 18 to 30, 18 to 50, 19 to 22, 19 to 30, 19 to 50, or 20 to 30 linked subunits. In certain such embodiments, the antisense compounds are 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked subunits in length, or a range defined by any two of the above values. In some embodiments the antisense compound is an antisense oligonucleotide, and the linked subunits are nucleotides.

In certain embodiments antisense oligonucleotides targeted to a HBV nucleic acid may be shortened or truncated. For example, a single subunit may be deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated antisense compound targeted to a HBV nucleic acid may have two subunits deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end, of the antisense compound. Alternatively, the deleted nucleosides may be dispersed throughout the antisense compound, for example, in an antisense compound having one nucleoside deleted from the 5' end and one nucleoside deleted from the 3' end.

When a single additional subunit is present in a lengthened antisense compound, the additional subunit may be located at the 5' or 3' end of the antisense compound. When two or more additional subunits are present, the added subunits may be adjacent to each other, for example, in an antisense compound having two subunits added to the 5' end (5' addition), or alternatively to the 3' end (3' addition), of the antisense compound. Alternatively, the added subunits may be dispersed throughout the antisense compound, for example, in an antisense compound having one subunit added to the 5' end and one subunit added to the 3' end.

It is possible to increase or decrease the length of an antisense compound, such as an antisense oligonucleotide, and/or introduce mismatch bases without eliminating activity. For example, in Woolf et al. (Proc. Natl. Acad. Sci. USA 89:7305-7309, 1992), a series of antisense oligonucleotides 13-25 nucleobases

in length were tested for their ability to induce cleavage of a target RNA in an oocyte injection model. Antisense oligonucleotides 25 nucleobases in length with 8 or 11 mismatch bases near the ends of the antisense oligonucleotides were able to direct specific cleavage of the target mRNA, albeit to a lesser extent than the antisense oligonucleotides that contained no mismatches. Similarly, target specific cleavage was achieved using 13 nucleobase antisense oligonucleotides, including those with 1 or 3 mismatches.

Gautschi *et al.* (*J. Natl. Cancer Inst.* 93:463-471, March 2001) demonstrated the ability of an oligonucleotide having 100% complementarity to the bcl-2 mRNA and having 3 mismatches to the bcl-xL mRNA to reduce the expression of both bcl-2 and bcl-xL *in vitro* and *in vivo*. Furthermore, this oligonucleotide demonstrated potent anti-tumor activity *in vivo*.

Maher and Dolnick (*Nuc. Acid. Res.* 16:3341-3358,1988) tested a series of tandem 14 nucleobase antisense oligonucleotides, and a 28 and 42 nucleobase antisense oligonucleotides comprised of the sequence of two or three of the tandem antisense oligonucleotides, respectively, for their ability to arrest translation of human DHFR in a rabbit reticulocyte assay. Each of the three 14 nucleobase antisense oligonucleotides alone was able to inhibit translation, albeit at a more modest level than the 28 or 42 nucleobase antisense oligonucleotides.

Antisense Compound Motifs

In certain embodiments, antisense compounds targeted to a HBV nucleic acid have chemically modified subunits arranged in patterns, or motifs, to confer to the antisense compounds properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by *in vivo* nucleases.

Chimeric antisense compounds typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, increased binding affinity for the target nucleic acid, and/or increased inhibitory activity. A second region of a chimeric antisense compound may optionally serve as a substrate for the cellular endonuclease RNase H, which cleaves the RNA strand of an RNA:DNA duplex.

Antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer an internal region having a plurality of nucleotides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified

nucleosides. In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β-D-ribonucleosides, β-D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE and 2'-O-CH_3, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a constrained ethyl). In certain embodiments, nucleosides in the wings may include several modified sugar moieties, including, for example 2'-MOE and bicyclic sugar moieties such as constrained ethyl or LNA. In certain embodiments, wings may include several modified and unmodified sugar moieties. In certain embodiments, wings may include various combinations of 2'-MOE nucleosides, bicyclic sugar moieties such as constrained ethyl nucleosides or LNA nucleosides, and 2'-deoxynucleosides.

Each distinct region may comprise uniform sugar moieties, variant, or alternating sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5'-wing, "Y" represents the length of the gap, and "Z" represents the length of the 3'-wing. "X" and "Z" may comprise uniform, variant, or alternating sugar moieties. In certain embodiments, "X" and "Y" may include one or more 2'-deoxynucleosides."Y" may comprise 2'-deoxynucleosides. As used herein, a gapmer described as "X-Y-Z" has a configuration such that the gap is positioned immediately adjacent to each of the 5'-wing and the 3' wing. Thus, no intervening nucleotides exist between the 5'-wing and gap, or the gap and the 3'-wing. Any of the antisense compounds described herein can have a gapmer motif. In certain embodiments, "X" and "Z" are the same; in other embodiments they are different. In certain embodiments, "Y" is between 8 and 15 nucleosides. X, Y, or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more nucleosides.

In certain embodiments, gapmers provided herein include, for example, 11-mers having a motif of 1-9-1.

In certain embodiments, gapmers provided herein include, for example, 12-mers having a motif of 1-9-2, 2-9-1, or 1-10-1.

In certain embodiments, gapmers provided herein include, for example, 13-mers having a motif of 1-9-3, 2-9-2, 3-9-1, 1-10-2, or 2-10-1.

In certain embodiments, gapmers provided herein include, for example, 14-mers having a motif of 1-9-4, 2-9-3, 3-9-2, 4-9-1, 1-10-3, 2-10-2, or 3-10-1.

In certain embodiments, gapmers provided herein include, for example, 15-mers having a motif of 1-9-5, 2-9-4, 3-9-3, 4-9-2, 5-9-1, 1-10-4, 2-10-3, 3-10-2, or 4-10-1.

In certain embodiments, gapmers provided herein include, for example, 16-mers having a motif of 4-8-4, 2-9-5, 3-9-4, 4-9-3, 5-9-2, 1-10-5, 2-10-4, 3-10-3, 4-10-2, 3-8-5, or 5-10-1.

In certain embodiments, gapmers provided herein include, for example, 17-mers having a motif of 3-9-5, 3-10-4, 4-9-4, 5-9-3, 2-10-5, 3-10-4, 4-10-3, 5-10-2, 2-9-6, 5-8-4, 5-7-5, 6-7-4, or 6-9-2.

In certain embodiments, gapmers provided herein include, for example, 18-mers having a motif of 4-9-5, 5-9-4, 3-10-5, 4-10-4, or 5-10-3.

In certain embodiments, gapmers provided herein include, for example, 19-mers having a motif of 5-9-5, 4-10-5, or 5-10-4.

In certain embodiments, gapmers provided herein include, for example, 20-mers having a motif of 5-10-5, 2-10-8, 8-10-2, 3-10-7, 7-10-3, 4-10-6, or 6-10-4.

In certain embodiments, the antisense compound has a "wingmer" motif, having a wing-gap or gap-wing configuration, i.e. an X-Y or Y-Z configuration as described above for the gapmer configuration. Thus, wingmer configurations provided herein include, but are not limited to, for example 5-10, 8-4, 4-12, 12-4, 3-14, 16-2, 18-1, 10-3, 2-10, 1-10, 8-2, 2-13, 5-13, 5-8, or 6-8.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 2-10-2 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 3-10-3 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 4-10-4 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 5-10-5 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 3-10-4 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 2-10-4 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 2-10-8 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 8-10-2 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 3-10-7 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 7-10-3 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 4-10-6 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 6-10-4 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 2-9-6 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 6-9-2 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 4-9-4 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 5-9-3 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 3-9-5 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 5-9-2 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 2-9-5 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 4-9-3 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a 3-9-4 gapmer motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a gap-widened motif.

In certain embodiments, the antisense compound targeted to a HBV nucleic acid has a gapmer motif in which the gap consists of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 linked nucleosides.

In certain embodiments, the antisense compounds targeted to a HBV nucleic acid has any of the following sugar motifs:

k-d(10)-k

e-d(10)-k

k-d(10)-e

k-k-d(10)-k-k

k-k-d(10)-e-e

e-e-d(10)-k-k

k-k-k-d(10)-k-k-k

e-e-e-d(10)-k-k-k

k-k-k-d(10)-e-e-e

k-k-k-d(10)-k-k-k

e-k-k-d(10)-k-k-e

e-e-k-d(10)-k-k-e

e-d-k-d(10)-k-k-e

e-k-d(10)-k-e-k-e

k-d(10)-k-e-k-e-e

e-e-k-d(10)-k-e-k-e

e-d-d-k-d(9)-k-k-e

e-e-e-e-d(9)-k-k-e

e-e-e-e-e-d(10)-e-e-e-e-e

k-d-k-d-k-d(9)-e-e

e-e-k-k-d(9)-e-k-e-e

k-d-k-d-k-d(10)-e-e-e-e-e

k-e-k-d(10)-k-e-k

e-e-e-k-k-d(8)-e-e-e-e

e-e-e-k-k-d(7)-k-k-e-e-e

e-e-e-k-d(9)-k-e-e-e

e-e-e-k-k-d(7)-k-k-e-e-e

e-e-e-e-k-k-d(7)-e-e-e-e

e-k-e-k-d(9)-e-e-e-e

e-k-e-k-d-k-d(7)-e-e-e-e

e-e-e-k-k-d(7)-k-k-e-e-e

k-d-k-d-k-d(8)-e-e-e-e-e

wherein, k is a constrained ethyl nucleoside, e is a 2'-MOE substituted nucleoside, and d is a 2'-deoxynucleoside.

In certain embodiments, the antisense oligonucleotide has a sugar motif described by Formula A as follows: $(J)_m$-$(B)_n$-$(J)_p$-$(B)_r$-$(A)_t$-$(D)_g$-$(A)_v$-$(B)_w$-$(J)_x$-$(B)_y$-$(J)_z$

wherein:

each A is independently a 2'-substituted nucleoside;

each B is independently a bicyclic nucleoside;

each J is independently either a 2'-substituted nucleoside or a 2'-deoxynucleoside;

each D is a 2'-deoxynucleoside;

m is 0-4; n is 0-2; p is 0-2; r is 0-2; t is 0-2; v is 0-2; w is 0-4; x is 0-2; y is 0-2; z is 0-4; g is 6-14;

provided that:

at least one of m, n, and r is other than 0;

at least one of w and y is other than 0;

the sum of m, n, p, r, and t is from 2 to 5; and

the sum of v, w, x, y, and z is from 2 to 5.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

Nucleotide sequences that encode HBV include, without limitation, the following: GENBANK Accession U95551.1 (incorporated herein as SEQ ID NO: 1).

It is understood that the sequence set forth in each SEQ ID NO in the Examples contained herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, antisense compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase. Antisense compounds described by Isis Number (Isis No) indicate a combination of nucleobase sequence and motif.

In certain embodiments, a target region is a structurally defined region of the target nucleic acid. For example, a target region may encompass a 3' UTR, a 5' UTR, an exon, an intron, an exon/intron junction, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for HBV can be obtained by accession number from sequence databases such as NCBI and such information is incorporated herein by reference. In certain embodiments, a target region may encompass the sequence from a 5' target site of one target segment within the target region to a 3' target site of another target segment within the same target region.

Targeting includes determination of at least one target segment to which an antisense compound hybridizes, such that a desired effect occurs. In certain embodiments, the desired effect is a reduction in mRNA target nucleic acid levels. In certain embodiments, the desired effect is reduction of levels of protein encoded by the target nucleic acid or a phenotypic change associated with the target nucleic acid.

A target region may contain one or more target segments. Multiple target segments within a target region may be overlapping. Alternatively, they may be non-overlapping. In certain embodiments, target segments within a target region are separated by no more than about 300 nucleotides. In certain emodiments, target segments within a target region are separated by a number of nucleotides that is, is about, is no more

than, is no more than about, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides on the target nucleic acid, or is a range defined by any two of the preceeding values. In certain embodiments, target segments within a target region are separated by no more than, or no more than about, 5 nucleotides on the target nucleic acid. In certain embodiments, target segments are contiguous. Contemplated are target regions defined by a range having a starting nucleic acid that is any of the 5' target sites or 3' target sites listed herein.

Suitable target segments may be found within a 5' UTR, a coding region, a 3' UTR, an intron, an exon, or an exon/intron junction. Target segments containing a start codon or a stop codon are also suitable target segments. A suitable target segment may specifcally exclude a certain structurally defined region such as the start codon or stop codon.

The determination of suitable target segments may include a comparison of the sequence of a target nucleic acid to other sequences throughout the genome. For example, the BLAST algorithm may be used to identify regions of similarity amongst different nucleic acids. This comparison can prevent the selection of antisense compound sequences that may hybridize in a non-specific manner to sequences other than a selected target nucleic acid (i.e., non-target or off-target sequences).

There may be variation in activity (e.g., as defined by percent reduction of target nucleic acid levels) of the antisense compounds within an active target region. In certain embodiments, reductions in HBV mRNA levels are indicative of inhibition of HBV expression. Reductions in levels of a HBV protein are also indicative of inhibition of target mRNA expression. Further, phenotypic changes are indicative of inhibition of HBV expression. In certain embodiments, reduced fatigue, reduced flu-like symptoms, increase in appetite, reduced nausea, reduced joint pain, reduced jaundice, reduced pain in the abdomen, reduced weakness, reduced weight loss, reduction in breast enlargement in men, reduced rash on the palms, reduced difficulty with blood clotting, reduced cirrhosis, reduced spider-like blood vessels on the skin, increased Vitamins A and D absorption, reduced tumor growth, reduced tumor volume, reduced headache, reduced fever, reduced diarrhea, reduced pain over the liver area of the body, reduced clay- or grey-colored stool, reduced itching, reduced dark-colored urine, and reduced nausea and vomiting can be indicative of inhibition of HBV expression, In certain embodiments, amelioration of symptoms associated with HBV-related conditions, disease, and disorders can be indicative of inhibition of HBV expression. In certain embodiments, reduction of cirrhosis is indicative of inhibition of HBV expression. In certain embodiments, reduction of liver cancer markers can be indicative of inhibition of HBV expression.

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and a HBV nucleic acid. The most common mechanism of hybridization involves hydrogen bonding (e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules.

Hybridization can occur under varying conditions. Stringent conditions are sequence-dependent and are determined by the nature and composition of the nucleic acid molecules to be hybridized.

Methods of determining whether a sequence is specifically hybridizable to a target nucleic acid are well known in the art. In certain embodiments, the antisense compounds provided herein are specifically hybridizable with a HBV nucleic acid.

Complementarity

An antisense compound and a target nucleic acid are complementary to each other when a sufficient number of nucleobases of the antisense compound can hydrogen bond with the corresponding nucleobases of the target nucleic acid, such that a desired effect will occur (e.g., antisense inhibition of a target nucleic acid, such as a HBV nucleic acid).

Non-complementary nucleobases between an antisense compound and a HBV nucleic acid may be tolerated provided that the antisense compound remains able to specifically hybridize to a target nucleic acid. Moreover, an antisense compound may hybridize over one or more segments of a HBV nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

In certain embodiments, the antisense compounds provided herein, or a specified portion thereof, are, or are at least, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to a HBV nucleic acid, a target region, target segment, or specified portion thereof. Percent complementarity of an antisense compound with a target nucleic acid can be determined using routine methods.

For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having four

noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul *et al., J. Mol. Biol.*, 1990, 215, 403 410; Zhang and Madden, Genome Res., 1997, 7, 649 656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482 489).

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, an antisense compound may be fully complementary to a HBV nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, "fully complementary" means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be "fully complementary" to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

The location of a non-complementary nucleobase may be at the 5' end or 3' end of the antisense compound. Alternatively, the non-complementary nucleobase or nucleobases may be at an internal position of the antisense compound. When two or more non-complementary nucleobases are present, they may be contiguous (i.e. linked) or non-contiguous. In one embodiment, a non-complementary nucleobase is located in the wing segment of a gapmer antisense oligonucleotide.

In certain embodiments, antisense compounds that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleobases in length comprise no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a HBV nucleic acid, or specified portion thereof.

In certain embodiments, antisense compounds that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a HBV nucleic acid, or specified portion thereof.

The antisense compounds provided also include those which are complementary to a portion of a target nucleic acid. As used herein, "portion" refers to a defined number of contiguous (i.e. linked) nucleobases within a region or segment of a target nucleic acid. A "portion" can also refer to a defined number of contiguous nucleobases of an antisense compound. In certain embodiments, the antisense compounds, are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 9 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 10 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least an 11 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 12 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 13 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 14 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 15 nucleobase portion of a target segment. Also contemplated are antisense compounds that are complementary to at least a 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nucleobase portion of a target segment, or a range defined by any two of these values.

Identity

The antisense compounds provided herein may also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific Isis number, or portion thereof. As used herein, an antisense compound is identical to the sequence disclosed herein if it has the same nucleobase pairing ability. For example, a RNA which contains uracil in place of thymidine in a disclosed DNA sequence would be considered identical to the DNA sequence since both uracil and thymidine pair with adenine. Shortened and lengthened versions of the antisense compounds described herein as well as compounds having non-identical bases relative to the antisense compounds provided herein also are contemplated. The non-identical bases may be adjacent to each other or dispersed throughout the antisense compound. Percent identity of an antisense compound is calculated according to the number of bases that have identical base pairing relative to the sequence to which it is being compared.

In certain embodiments, the antisense compounds, or portions thereof, are at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to one or more of the antisense compounds or SEQ ID NOs, or a portion thereof, disclosed herein.

In certain embodiments, a portion of the antisense compound is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

In certain embodiments, a portion of the antisense oligonucleotide is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

Modifications

A nucleoside is a base-sugar combination. The nucleobase (also known as base) portion of the nucleoside is normally a heterocyclic base moiety. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', 3' or 5' hydroxyl moiety of the sugar. Oligonucleotides are formed through the covalent linkage of adjacent nucleosides to one another, to form a linear polymeric oligonucleotide. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide.

Modifications to antisense compounds encompass substitutions or changes to internucleoside linkages, sugar moieties, or nucleobases. Modified antisense compounds are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target, increased stability in the presence of nucleases, or increased inhibitory activity.

Chemically modified nucleosides may also be employed to increase the binding affinity of a shortened or truncated antisense oligonucleotide for its target nucleic acid. Consequently, comparable results can often be obtained with shorter antisense compounds that have such chemically modified nucleosides.

Modified Internucleoside Linkages

The naturally occuring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. Antisense compounds having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over antisense compounds having naturally occurring internucleoside linkages

because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target nucleic acids, and increased stability in the presence of nucleases.

Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters, phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

In certain embodiments, antisense compounds targeted to a HBV nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

Modified Sugar Moieties

Antisense compounds provided herein can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise a chemically modified ribofuranose ring moiety. Examples of chemically modified ribofuranose rings include, without limitation, addition of substitutent groups (including 5' and 2' substituent groups); bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA); replacement of the ribosyl ring oxygen atom with S, N(R), or C(R1)(R)2 (R = H, C_1-C_{12} alkyl or a protecting group); and combinations thereof. Examples of chemically modified sugars include, 2'-F-5'-methyl substituted nucleoside (*see*, PCT International Application WO 2008/101157, published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides), replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (*see*, published U.S. Patent Application US2005/0130923, published on June 16, 2005), or, alternatively, 5'-substitution of a BNA (*see*, PCT International Application WO 2007/134181, published on 11/22/07, wherein LNA is substituted with, for example, a 5'-methyl or a 5'-vinyl group).

Examples of nucleosides having modified sugar moieties include, without limitation, nucleosides comprising 5'-vinyl, 5'-methyl (R or S), 4'-S, 2'-F, 2'-OCH_3, and 2'-$O(CH_2)2OCH_3$ substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, OCF_3, $O(CH_2)2SCH_3$, $O(CH_2)2$-O-N(Rm)(Rn), and O-CH_2-C(=O)-N(Rm)(Rn), where each Rm and Rn is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl.

As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleosides include, without limitation, nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more bicyclic nucleosides wherein the bridge comprises a 4' to 2' bicyclic nucleoside. Examples of such 4' to 2' bicyclic nucleosides, include, but are not limited to, one of the formulae: 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)-S-2'; 4'-$(CH_2)_2$-O-2' (ENA); 4'-$CH(CH_3)$-O-2' (cEt) and 4'-$CH(CH_2OCH_3)$-O-2', and analogs thereof (*see*, U.S. Patent 7,399,845, issued on July 15, 2008); 4'-$C(CH_3)(CH_3)$-O-2', and analogs thereof (*see*, published PCT International Application WO2009/006478, published January 8, 2009); 4'-CH_2-$N(OCH_3)$-2', and analogs thereof (*see*, published PCT International Application WO2008/150729, published December 11, 2008); 4'-CH_2-O-$N(CH_3)$-2' (*see*, published U.S. Patent Application US2004/0171570, published September 2, 2004); 4'-CH_2-N(R)-O-2', wherein R is H, C_1-C_{12} alkyl, or a protecting group (*see*, U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH_2-$C(H)(CH_3)$-2' (*see*, Chattopadhyaya, *et al., J. Org. Chem.*,2009, *74*, 118-134); and 4'-CH_2-$C(=CH_2)$-2', and analogs thereof (*see*, published PCT International Application WO 2008/154401, published on December 8, 2008). Also see, for example: Singh et al., *Chem. Commun.*, 1998, *4*, 455-456; Koshkin et al., *Tetrahedron*, 1998, *54*, 3607-3630; Wahlestedt et al., *Proc. Natl. Acad. Sci. U. S. A.*, 2000, *97*, 5633-5638; Kumar et al., *Bioorg. Med. Chem. Lett.*, 1998, *8*, 2219-2222; Singh et al., *J. Org. Chem.*, 1998, *63*, 10035-10039; Srivastava et al., *J. Am. Chem. Soc.*, *129(26)* 8362-8379 (Jul. 4, 2007); Elayadi *et al., Curr. Opinion Invens. Drugs*, 2001, *2*, 558-561; Braasch *et al., Chem. Biol.*, 2001, *8*, 1-7; Orum *et al., Curr. Opinion Mol. Ther.*, 2001, *3*, 239-243; U.S. Patent Nos U.S. 6,670,461, 7,053,207, 6,268,490, 6,770,748, 6,794,499, 7,034,133, 6,525,191, 7,399,845; published PCT International applications WO 2004/106356, WO 94/14226, WO 2005/021570, and WO 2007/134181; U.S. Patent Publication Nos. US2004/0171570, US2007/0287831, and US2008/0039618; and U.S. Patent Serial Nos. 12/129,154, 60/989,574, 61/026,995, 61/026,998, 61/056,564, 61/086,231, 61/097,787, and 61/099,844; and PCT International Application Nos. PCT/US2008/064591, PCT/US2008/066154, and PCT/US2008/068922. Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

In certain embodiments, bicyclic sugar moieties of BNA nucleosides include, but are not limited to, compounds having at least one bridge between the 4' and the 2' position of the pentofuranosyl sugar moiety wherein such bridges independently comprises 1 or from 2 to 4 linked groups independently selected from -$[C(R_a)(R_b)]_n$-, -$C(R_a)=C(R_b)$-, -$C(R_a)=N$-, -$C(=NR_a)$-, -C(=O)-, -C(=S)-, -O-, -$Si(R_a)_2$-, -$S(=O)_x$-, and -$N(R_a)$-;

wherein:

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

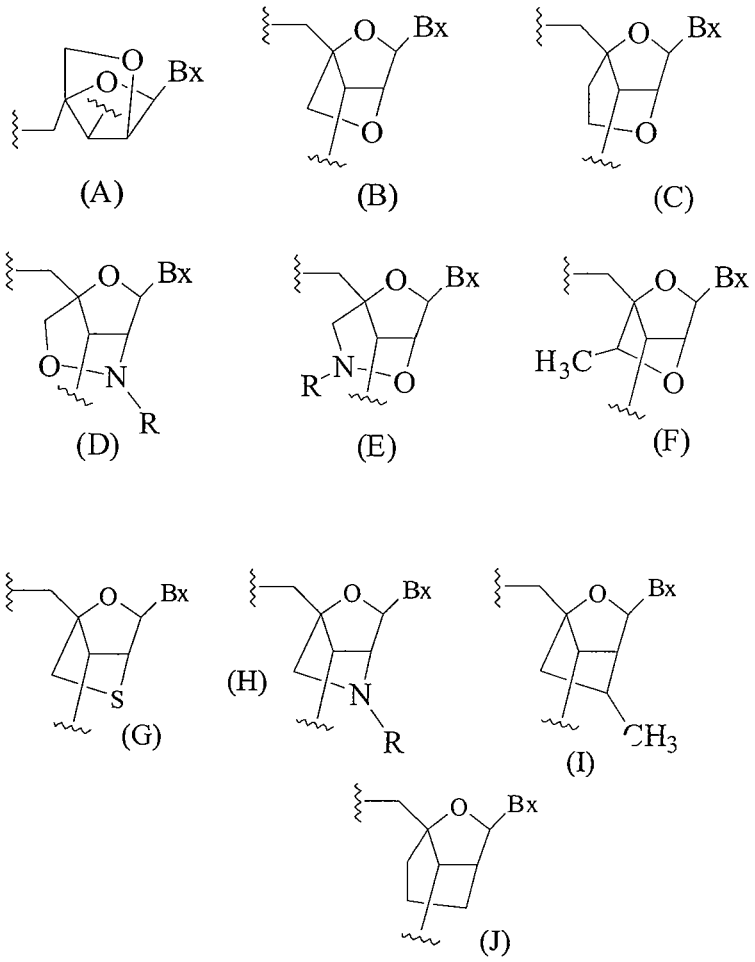
each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5-C_7 alicyclic radical, substituted C_5-C_7 alicyclic radical, halogen, OJ_1, NJ_1J_2, SJ_1, N_3, $COOJ_1$, acyl (C(=O)-H), substituted acyl, CN, sulfonyl ($S(=O)_2$-J_1), or sulfoxyl (S(=O)-J_1); and

each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1-C_{12} aminoalkyl, substituted C_1-C_{12} aminoalkyl, or a protecting group.

In certain embodiments, the bridge of a bicyclic sugar moiety is, -$[C(R_a)(R_b)]_n$-, -$[C(R_a)(R_b)]_n$-O-, -$C(R_aR_b)$-N(R)-O- or, –$C(R_aR_b)$-O-N(R)-. In certain embodiments, the bridge is 4'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-$(CH_2)_3$-2', 4'-CH_2-O-2', 4'-$(CH_2)_2$-O-2', 4'-CH_2-O-N(R)-2', and 4'-CH_2-N(R)-O-2'-, wherein each R is, independently, H, a protecting group, or C_1-C_{12} alkyl.

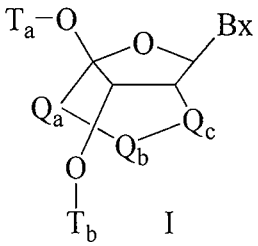
In certain embodiments, bicyclic nucleosides are further defined by isomeric configuration. For example, a nucleoside comprising a 4'-2' methylene-oxy bridge, may be in the α-L configuration or in the β-D configuration. Previously, α-L-methyleneoxy (4'-CH_2-O-2') BNA's have been incorporated into antisense oligonucleotides that showed antisense activity (Frieden *et al., Nucleic Acids Research,* 2003, *21*, 6365-6372).

In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α-L-Methyleneoxy (4'-CH_2-O-2') BNA , (B) β-D-Methyleneoxy (4'-CH_2-O-2') BNA , (C) Ethyleneoxy (4'-$(CH_2)_2$-O-2') BNA , (D) Aminooxy (4'-CH_2-O-N(R)-2') BNA, (E) Oxyamino (4'-CH_2-N(R)-O-2') BNA, (F) Methyl(methyleneoxy) (4'-$CH(CH_3)$-O-2') BNA, (G) methylene-thio (4'-CH_2-S-2') BNA, (H) methylene-amino (4'-CH2-N(R)-2') BNA, (I) methyl carbocyclic (4'-CH_2-$CH(CH_3)$-2') BNA, and (J) propylene carbocyclic (4'-$(CH_2)_3$-2') BNA as depicted below.



wherein Bx is the base moiety and R is, independently, H, a protecting group or C_1-C_{12} alkyl.

In certain embodiments, bicyclic nucleoside having Formula I:



I

wherein:

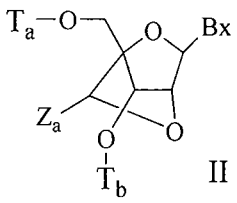
Bx is a heterocyclic base moiety;

$-Q_a-Q_b-Q_c-$ is $-CH_2-N(R_c)-CH_2-$, $-C(=O)-N(R_c)-CH_2-$, $-CH_2-O-N(R_c)-$, $-CH_2-N(R_c)-O-$, or $-N(R_c)-O-CH_2$;

R_c is C_1-C_{12} alkyl or an amino protecting group; and

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium.

In certain embodiments, bicyclic nucleoside having Formula II:



II

wherein:

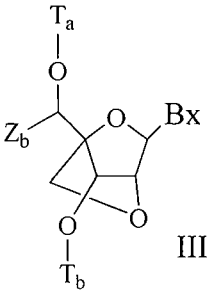
Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

Z_a is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_1-C_6 alkyl, substituted C_2-C_6 alkenyl, substituted C_2-C_6 alkynyl, acyl, substituted acyl, substituted amide, thiol, or substituted thio.

In one embodiment, each of the substituted groups is, independently, mono or poly substituted with substituent groups independently selected from halogen, oxo, hydroxyl, OJ_c, NJ_cJ_d, SJ_c, N_3, $OC(=X)J_c$, and $NJ_eC(=X)NJ_cJ_d$, wherein each J_c, J_d, and J_e is, independently, H, C_1-C_6 alkyl, or substituted C_1-C_6 alkyl and X is O or NJ_c.

In certain embodiments, bicyclic nucleoside having Formula III:



III

wherein:

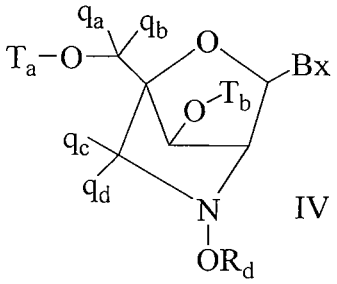
Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

Z_b is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_1-C_6 alkyl, substituted C_2-C_6 alkenyl,

substituted C_2-C_6 alkynyl, or substituted acyl (C(=O)-).

In certain embodiments, bicyclic nucleoside having Formula IV:



IV

wherein:

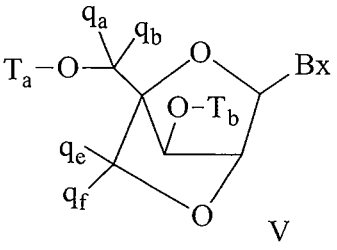
Bx is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

R_d is C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, or substituted C_2-C_6 alkynyl;

each q_a, q_b, q_c and q_d is, independently, H, halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, or substituted C_2-C_6 alkynyl, C_1-C_6 alkoxyl, substituted C_1-C_6 alkoxyl, acyl, substituted acyl, C_1-C_6 aminoalkyl, or substituted C_1-C_6 aminoalkyl;

In certain embodiments, bicyclic nucleoside having Formula V:



V

wherein:

Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

q_a, q_b, q_e and q_f are each, independently, hydrogen, halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, substituted C_1-C_{12} alkoxy, OJ_j, SJ_j, SOJ_j, SO_2J_j, NJ_jJ_k, N_3, CN, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O\text{-}C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ or $N(H)C(=S)NJ_jJ_k$;

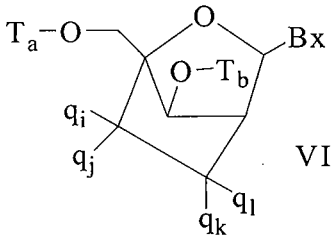
or q_e and q_f together are $=C(q_g)(q_h)$;

q_g and q_h are each, independently, H, halogen, C_1-C_{12} alkyl, or substituted C_1-C_{12} alkyl.

The synthesis and preparation of the methyleneoxy (4'-CH_2-O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine, and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (*see, e.g.*, Koshkin et al., *Tetrahedron*, 1998, *54*, 3607-3630). BNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

Analogs of methyleneoxy (4'-CH_2-O-2') BNA, methyleneoxy (4'-CH_2-O-2') BNA, and 2'-thio-BNAs, have also been prepared (*see, e.g.*, Kumar et al., *Bioorg. Med. Chem. Lett.*, 1998, *8*, 2219-2222). Preparation of locked nucleoside analogs comprising oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (*see, e.g.*, Wengel et al., WO 99/14226). Furthermore, synthesis of 2'-amino-BNA, a novel comformationally restricted high-affinity oligonucleotide analog, has been described in the art (*see, e.g.*, Singh et al., *J. Org. Chem.*, 1998, *63*, 10035-10039). In addition, 2'-amino- and 2'-methylamino-BNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

In certain embodiments, bicyclic nucleoside having Formula VI:



VI

wherein:

Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

each q_i, q_j, q_k and q_l is, independently, H, halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxyl, substituted C_1-C_{12} alkoxyl, OJ_j, SJ_j, SOJ_j, SO_2J_j, NJ_jJ_k, N_3, CN, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O\text{-}C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$, or $N(H)C(=S)NJ_jJ_k$; and

q_i and q_j or q_l and q_k together are $=C(q_g)(q_h)$, wherein q_g and q_h are each, independently, H, halogen, C_1-C_{12} alkyl, or substituted C_1-C_{12} alkyl.

One carbocyclic bicyclic nucleoside having a 4'-$(CH_2)_3$-2' bridge and the alkenyl analog, bridge 4'-CH=CH-CH_2-2', have been described (*see, e.g.*, Freier *et al., Nucleic Acids Research*, 1997, *25(22)*, 4429-4443 and Albaek *et al., J. Org. Chem.*, 2006, *71*, 7731-7740). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (*see, e.g.*, Srivastava *et al., J. Am. Chem. Soc.* 2007, *129(26)*, 8362-8379).

As used herein, "bicyclic nucleoside" refers to a nucleoside comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic sugar moiety. In certain embodiments, the bridge connects the 2' carbon and another carbon of the sugar ring.

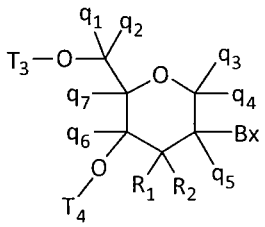
As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting the 2' carbon atom and the 4' carbon atom.

As used herein, "monocylic nucleosides" refer to nucleosides comprising modified sugar moieties that are not bicyclic sugar moieties. In certain embodiments, the sugar moiety, or sugar moiety analogue, of a nucleoside may be modified or substituted at any position.

As used herein, "2'-modified sugar" means a furanosyl sugar modified at the 2' position. In certain embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and unsubstituted amino alkyl, substituted and unsubstituted alkyl, substituted and unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2' modifications are selected from substituents including, but not limited to: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. Other 2'- substituent groups can also be selected from: C_1-C_{12} alkyl; substituted alkyl; alkenyl; alkynyl; alkaryl; aralkyl; O-alkaryl or O-aralkyl; SH; SCH_3; OCN; Cl; Br; CN; CF_3; OCF_3; $SOCH_3$; SO_2CH_3; ONO_2; NO_2; N_3; NH_2; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving pharmacokinetic properties; and a group for improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modifed nucleosides comprise a 2'-MOE side chain (*see, e.g.*, Baker et al., J. Biol. Chem., 1997, 272, 11944-12000). Such 2'-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2'- *O*-methyl, *O*-propyl, and *O*-aminopropyl. Oligonucleotides having the 2'-MOE substituent also have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (*see, e.g.*, Martin, P., *Helv. Chim. Acta*, 1995, *78*, 486-504; Altmann et al., *Chimia*, 1996, *50*, 168-176; Altmann et al., *Biochem. Soc. Trans.*, 1996, *24*, 630-637; and Altmann et al., *Nucleosides Nucleotides*, 1997, *16*, 917-926).

As used herein, a "modified tetrahydropyran nucleoside" or "modified THP nucleoside" means a nucleoside having a six-membered tetrahydropyran "sugar" substituted in for the pentofuranosyl residue in normal nucleosides (a sugar surrogate). Modified THP nucleosides include, but are not limited to, what is referred to in the art as hexitol nucleic acid (HNA), anitol nucleic acid (ANA), manitol nucleic acid (MNA) (*see* Leumann, CJ. *Bioorg. & Med. Chem.* (2002) **10**:841-854), fluoro HNA (F-HNA), or those compounds having Formula X:

Formula X:



X

wherein independently for each of said at least one tetrahydropyran nucleoside analog of Formula X:

Bx is a heterocyclic base moiety;

T_3 and T_4 are each, independently, an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound or one of T_3 and T_4 is an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound and the other of T_3 and T_4 is H, a hydroxyl protecting group, a linked conjugate group, or a 5' or 3'-terminal group;

q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each, independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, or substituted C_2-C_6 alkynyl; and

one of R_1 and R_2 is hydrogen and the other is selected from halogen, substituted or unsubstituted alkoxy, NJ_1J_2, SJ_1, N_3, $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ_3C(=X)NJ_1J_2$, and CN, wherein X is O, S, or NJ_1, and each J_1, J_2, and J_3 is, independently, H or C_1-C_6 alkyl.

In certain embodiments, the modified THP nucleosides of Formula X are provided wherein q_m, q_n, q_p, q_r, q_s, q_t, and q_u are each H. In certain embodiments, at least one of q_m, q_n, q_p, q_r, q_s, q_t, and q_u is other than H. In certain embodiments, at least one of q_m, q_n, q_p, q_r, q_s, q_t and q_u is methyl. In certain embodiments, THP nucleosides of Formula X are provided wherein one of R_1 and R_2 is F. In certain embodiments, R_1 is fluoro and R_2 is H, R_1 is methoxy and R_2 is H, and R_1 is methoxyethoxy and R_2 is H.

As used herein, "2'-modified nucleoside" or "2'-substituted nucleoside" refers to a nucleoside comprising a sugar comprising a substituent at the 2' position of a furanose ring other than H or OH. 2'-modified nucleosides, include, but are not limited to, bicyclic nucleosides wherein the bridge connecting two carbon atoms of the sugar ring connects the 2' carbon and another carbon of the sugar ring and nucleosides with non-bridging 2'substituents, such as allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, -OCF_3, O-$(CH_2)_2$-O-CH_3, 2'-$O(CH_2)_2SCH_3$, O-$(CH_2)_2$-O-$N(R_m)(R_n)$, or O-CH_2-C(=O)-$N(R_m)(R_n)$, where each R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl. 2'-modifed nucleosides may further comprise other modifications, for example, at other positions of the sugar and/or at the nucleobase.

As used herein, "2'-F" refers to a sugar comprising a fluoro group at the 2' position.

As used herein, "2'-OMe" or "2'-OCH_3" or "2'-O-methyl" each refers to a nucleoside comprising a sugar comprising an -OCH_3 group at the 2' position of the sugar ring.

As used herein, "oligonucleotide" refers to a compound comprising a plurality of linked nucleosides. In certain embodiments, one or more of the plurality of nucleosides is modified. In certain embodiments, an oligonucleotide comprises one or more ribonucleosides (RNA) and/or deoxyribonucleosides (DNA).

Many other bicyclo and tricyclo sugar surrogate ring systems are also known in the art that can be used to modify nucleosides for incorporation into antisense compounds (*see, e.g.*, review article: Leumann, J. C, *Bioorganic & Medicinal Chemistry*, **2002**, *10*, 841-854). Such ring systems can undergo various additional substitutions to enhance activity.

Methods for the preparations of modified sugars are well known to those skilled in the art.

In nucleotides having modified sugar moieties, the nucleobase moieties (natural, modified, or a combination thereof) are maintained for hybridization with an appropriate nucleic acid target.

In certain embodiments, antisense compounds comprise one or more nucleotides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleotides are arranged in a gapmer motif. In certain embodiments, the modified sugar moiety is a cEt. In certain embodiments, the cEt modified nucleotides are arranged throughout the wings of a gapmer motif.

Compositions and Methods for Formulating Pharmaceutical Compositions

Antisense oligonucleotides may be admixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

An antisense compound targeted to a HBV nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable for use in compositions to be delivered parenterally. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to a HBV nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is PBS. In certain embodiments, the antisense compound is an antisense oligonucleotide.

Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite

or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

A prodrug can include the incorporation of additional nucleosides at one or both ends of an antisense compound which are cleaved by endogenous nucleases within the body, to form the active antisense compound.

Conjugated Antisense compounds

Antisense compounds may be covalently linked to one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the resulting antisense oligonucleotides. Typical conjugate groups include cholesterol moieties and lipid moieties. Additional conjugate groups include carbohydrates, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes.

Antisense compounds can also be modified to have one or more stabilizing groups that are generally attached to one or both termini of antisense compounds to enhance properties such as, for example, nuclease stability. Included in stabilizing groups are cap structures. These terminal modifications protect the antisense compound having terminal nucleic acid from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap), or at the 3'-terminus (3'-cap), or can be present on both termini. Cap structures are well known in the art and include, for example, inverted deoxy abasic caps. Further 3' and 5'-stabilizing groups that can be used to cap one or both ends of an antisense compound to impart nuclease stability include those disclosed in WO 03/004602 published on January 16, 2003.

Cell culture and antisense compounds treatment

The effects of antisense compounds on the level, activity or expression of HBV nucleic acids can be tested *in vitro* in a variety of cell types. Cell types used for such analyses are available from commerical vendors (*e.g.* American Type Culture Collection, Manassus, VA; Zen-Bio, Inc., Research Triangle Park, NC; Clonetics Corporation, Walkersville, MD) and are cultured according to the vendor's instructions using commercially available reagents (e.g. Invitrogen Life Technologies, Carlsbad, CA). Illustrative cell types

include, but are not limited to, HuVEC cells, b.END cells, HepG2 cells, Hep3B cells, and primary hepatocytes.

In vitro testing of antisense oligonucleotides

Described herein are methods for treatment of cells with antisense oligonucleotides, which can be modified appropriately for treatment with other antisense compounds.

Cells may be treated with antisense oligonucleotides when the cells reach approximately 60-80% confluency in culture.

One reagent commonly used to introduce antisense oligonucleotides into cultured cells includes the cationic lipid transfection reagent LIPOFECTIN (Invitrogen, Carlsbad, CA). Antisense oligonucleotides may be mixed with LIPOFECTIN in OPTI-MEM 1 (Invitrogen, Carlsbad, CA) to achieve the desired final concentration of antisense oligonucleotide and a LIPOFECTIN concentration that may range from 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

Another reagent used to introduce antisense oligonucleotides into cultured cells includes LIPOFECTAMINE (Invitrogen, Carlsbad, CA). Antisense oligonucleotide is mixed with LIPOFECTAMINE in OPTI-MEM 1 reduced serum medium (Invitrogen, Carlsbad, CA) to achieve the desired concentration of antisense oligonucleotide and a LIPOFECTAMINE concentration that may range from 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

Another technique used to introduce antisense oligonucleotides into cultured cells includes electroporation.

Cells are treated with antisense oligonucleotides by routine methods. Cells may be harvested 16-24 hours after antisense oligonucleotide treatment, at which time RNA or protein levels of target nucleic acids are measured by methods known in the art and described herein. In general, when treatments are performed in multiple replicates, the data are presented as the average of the replicate treatments.

The concentration of antisense oligonucleotide used varies from cell line to cell line. Methods to determine the optimal antisense oligonucleotide concentration for a particular cell line are well known in the art. Antisense oligonucleotides are typically used at concentrations ranging from 1 nM to 300 nM when transfected with LIPOFECTAMINE. Antisense oligonucleotides are used at higher concentrations ranging from 625 to 20,000 nM when transfected using electroporation.

RNA Isolation

RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. RNA is prepared using methods well known in the art, for example, using the TRIZOL Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's recommended protocols.

Analysis of inhibition of target levels or expression

Inhibition of levels or expression of a HBV nucleic acid can be assayed in a variety of ways known in the art. For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or quantitaive real-time PCR. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Quantitative real-time PCR can be conveniently accomplished using the commercially available ABI PRISM 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

Quantitative Real-Time PCR Analysis of Target RNA Levels

Quantitation of target RNA levels may be accomplished by quantitative real-time PCR using the ABI PRISM 7600, 7700, or 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Methods of quantitative real-time PCR are well known in the art.

Prior to real-time PCR, the isolated RNA is subjected to a reverse transcriptase (RT) reaction, which produces complementary DNA (cDNA) that is then used as the substrate for the real-time PCR amplification. The RT and real-time PCR reactions are performed sequentially in the same sample well. RT and real-time PCR reagents may be obtained from Invitrogen (Carlsbad, CA). RT real-time-PCR reactions are carried out by methods well known to those skilled in the art.

Gene (or RNA) target quantities obtained by real time PCR are normalized using either the expression level of a gene whose expression is constant, such as cyclophilin A, or by quantifying total RNA using RIBOGREEN (Invitrogen, Inc. Carlsbad, CA). Cyclophilin A expression is quantified by real time PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RIBOGREEN RNA quantification reagent (Invetrogen, Inc. Eugene, OR). Methods of RNA quantification by RIBOGREEN are taught in Jones, L.J., et al, (Analytical Biochemistry, 1998, 265, 368-374). A CYTOFLUOR 4000 instrument (PE Applied Biosystems) is used to measure RIBOGREEN fluorescence.

Probes and primers are designed to hybridize to a HBV nucleic acid. Methods for designing real-time PCR probes and primers are well known in the art, and may include the use of software such as PRIMER EXPRESS Software (Applied Biosystems, Foster City, CA).

Quantitative Real-Time PCR Analysis of Target DNA Levels

Quantitation of target DNA levels may be accomplished by quantitative real-time PCR using the ABI PRISM 7600, 7700, or 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Methods of quantitative real-time PCR are well known in the art.

Gene (or DNA) target quantities obtained by real time PCR are normalized using either the expression level of a gene whose expression is constant, such as cyclophilin A, or by quantifying total DNA using RIBOGREEN (Invitrogen, Inc. Carlsbad, CA). Cyclophilin A expression is quantified by real time PCR, by being run simultaneously with the target, multiplexing, or separately. Total DNA is quantified using RIBOGREEN RNA quantification reagent (Invetrogen, Inc. Eugene, OR). Methods of DNA quantification by RIBOGREEN are taught in Jones, L.J., et al, (Analytical Biochemistry, 1998, 265, 368-374). A CYTOFLUOR 4000 instrument (PE Applied Biosystems) is used to measure RIBOGREEN fluorescence.

Probes and primers are designed to hybridize to a HBV nucleic acid. Methods for designing real-time PCR probes and primers are well known in the art, and may include the use of software such as PRIMER EXPRESS Software (Applied Biosystems, Foster City, CA).

Analysis of Protein Levels

Antisense inhibition of HBV nucleic acids can be assessed by measuring HBV protein levels. Protein levels of HBV can be evaluated or quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art.

In vivo testing of antisense compounds

Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to inhibit expression of HBV and produce phenotypic changes. Testing may be performed in normal animals, or in experimental disease models. For administration to animals, antisense oligonucleotides are formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline. Administration includes parenteral routes of administration, such as intraperitoneal, intravenous, subcutaneous, intrathecal, and intracerebroventricular. Calculation of antisense oligonucleotide dosage and dosing frequency is within the abilities of those skilled in the art, and depends upon factors such as route of administration and animal body weight. Following a period of treatment with antisense oligonucleotides, RNA is isolated from liver tissue and changes in HBV nucleic acid expression are measured. Changes in HBV DNA levels are also measured. Changes in HBV protein levels are also measured. Changes in HBV HBeAg levels are also measured. Changes in HBV HBsAg levels are also measured.

Certain Indications

In certain embodiments, provided herein are methods, compounds, and compositions of treating an individual comprising administering one or more pharmaceutical compositions provided herein. In certain embodiments, the individual has an HBV-related condition. In certain embodiments, chronic HBV infection, inflammation, fibrosis, cirrhosis, liver cancer, serum hepatitis, jaundice, liver cancer, liver inflammation, liver fibrosis, liver cirrhosis, liver failure, diffuse hepatocellular inflammatory disease, hemophagocytic syndrome, serum hepatitis, and HBV viremia. In certain embodiments, the HBV-related condition may have which may include any or all of the following: flu-like illness, weakness, aches, headache, fever, loss of appetite, diarrhea, jaundice, nausea and vomiting, pain over the liver area of the body, clay- or grey-colored stool, itching all over, and dark-colored urine, when coupled with a positive test for presence of a hepatitis B virus, a hepatitis B viral antigen, or a positive test for the presence of an antibody specific for a hepatitis B viral antigen. In certain embodiments, the individual is at risk for an HBV-related condition. This includes individuals having one or more risk factors for developing an HBV-related condition, including sexual exposure to an individual infected with Hepatitis B virus, living in the same house as an individual with a lifelong hepatitis B virus infection, exposure to human blood infected with the hepatitis B virus, injection of illicit drugs, being a person who has hemophilia, and visiting an area where hepatitis B is common. In certain embodiments, the individual has been identified as in need of treatment for an HBV-related condition. In certain embodiments provided herein are methods for prophylactically reducing HBV expression in an individual. Certain embodiments include treating an individual in need thereof by administering to an individual a therapeutically effective amount of an antisense compound targeted to an HBV nucleic acid.

Due to overlapping transmission routes, many people have been exposed to both hepatitis B virus (HBV) and hepatitis C virus (HCV), and a smaller proportion are chronically infected with both viruses, especially in regions such as Asia where HBV is endemic. Estimates suggest that up to 10% of people with HCV may also have HBV, while perhaps 20% of people with HBV are co-infected with HCV. However, treatment of hepatitis B or hepatitis B in HBV-HCV co-infected individuals has not been well studied. Treatment is complicated by the fact that HCV and HBV appear to inhibit each other's replication (though not all studied have observed this interaction). Therefore, treatment that fully suppresses HBV could potentially allow HCV to re-emerge, or vice versa. Therefore, the compounds and compositions described herein may advantageously be used for treating patients infected with both HBV and HCV. Exemplary treatment options for hepatitis C (HCV) include interferons, e.g., interferon alpha-2b, interferon alpha-2a, and interferon alphacon-1. Less frequent interferon dosing can be achieved using pegylated interferon (interferon attached to a polyethylene glycol moiety which improves its pharmacokinetic profile). Combination therapy with interferon alpha-2b (pegylated and unpegylated) and ribavirin has also been shown to be efficacious for some patient populations. Other agents currently being developed include HCV RNA replication inhibitors (e.g., ViroPharma's VP50406 series), HCV antisense agents, HCV therapeutic vaccines, HCV protease inhibitors, HCV helicase inhibitors and HCV antibody therapy (monoclonal or polyclonal).

In certain embodiments, treatment with the methods, compounds, and compositions described herein is useful for preventing an HBV-related condition associated with the presence of the hepatitis B virus. In certain embodiments, treatment with the methods, compounds, and compositions described herein is useful for preventing an HBV-related condition.

In one embodiment, administration of a therapeutically effective amount of an antisense compound targeted to an HBV nucleic acid is accompanied by monitoring of HBV mRNA levels in the serum of an individual to determine an individual's response to administration of the antisense compound. In certain embodiments, administration of a therapeutically effective amount of an antisense compound targeted to an HBV nucleic acid is accompanied by monitoring of HBV DNA levels in the serum of an individual to determine an individual's response to administration of the antisense compound. In certain embodiments, administration of a therapeutically effective amount of an antisense compound targeted to an HBV nucleic acid is accompanied by monitoring of HBV protein levels in the serum of an individual to determine an individual's response to administration of the antisense compound. In certain embodiments, administration of a therapeutically effective amount of an antisense compound targeted to an HBV nucleic acid is accompanied by monitoring of HBV S antigen (HBsAg) levels in the serum of an individual to determine an individual's response to administration of the antisense compound. In certain embodiments, administration of a therapeutically effective amount of an antisense compound targeted to an HBV nucleic acid is accompanied by monitoring of HBV E antigen (HBeAg) levels in the serum of an individual to determine an individual's

response to administration of the antisense compound. An individual's response to administration of the antisense compound is used by a physician to determine the amount and duration of therapeutic intervention.

In certain embodiments, administration of an antisense compound targeted to an HBV nucleic acid results in reduction of HBV expression by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In certain embodiments, administration of an antisense compound targeted to an HBV nucleic acid results in reduced symptoms associated with the HBV-related condition and reduced HBV-related markers in the blood. In certain embodiments, administration of an HBV antisense compound decreases HBV RNA levels, HBV DNA levels, HBV protein levels, HBsAg levels, or HBeAg levels by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values.

In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to HBV are used for the preparation of a medicament for treating a patient suffering or susceptible to an HBV-related condition.

Certain Combination Therapies

In certain embodiments, one or more pharmaceutical compositions provided herein are co-administered with one or more other pharmaceutical agents. In certain embodiments, such one or more other pharmaceutical agents are designed to treat the same disease, disorder, or condition as the one or more pharmaceutical compositions provided herein. In certain embodiments, such one or more other pharmaceutical agents are designed to treat a different disease, disorder, or condition as the one or more pharmaceutical compositions provided herein. In certain embodiments, such one or more other pharmaceutical agents are designed to treat an undesired side effect of one or more pharmaceutical compositions provided herein. In certain embodiments, one or more pharmaceutical compositions provided herein are co-administered with another pharmaceutical agent to treat an undesired effect of that other pharmaceutical agent. In certain embodiments, one or more pharmaceutical compositions provided herein are co-administered with another pharmaceutical agent to produce a combinational effect. In certain embodiments, one or more pharmaceutical compositions provided herein are co-administered with another pharmaceutical agent to produce a synergistic effect.

In certain embodiments, one or more pharmaceutical compositions provided herein and one or more other pharmaceutical agents are administered at the same time. In certain embodiments, one or more pharmaceutical compositions provided herein and one or more other pharmaceutical agents are administered at different times. In certain embodiments, one or more pharmaceutical compositions provided herein and

one or more other pharmaceutical agents are prepared together in a single formulation. In certain embodiments, one or more pharmaceutical compositions provided herein and one or more other pharmaceutical agents are prepared separately. In certain embodiments the antisense oligonucleotides disclosed is administered in combination with an HCV agent. In further embodiments, the HCV compound is administered simultaneously as the antisense compound; in other embodiments, the HCV compound is administered separately; so that a dose of each of the HCV agent and the antisense compound overlap, in time, within the patient's body. In related embodiments, the HCV agent may be selected from interferon alpha-2b, interferon alpha-2a, and interferon alphacon-1 (pegylated and unpegylated); ribavirin; an HCV RNA replication inhibitor (e.g., ViroPharma's VP50406 series); an HCV antisense agent; an HCV therapeutic vaccine; an HCV protease inhibitor; an HCV helicase inhibitor; and an HCV antibody therapy (monoclonal or polyclonal).

In other embodiments, an HBV antisense compound of the present invention may be administered to a patient infected with HBV, in combination with one or more HBV therapeutic agents, wherein the one or more HBV therapeutic agents may be administered in the same drug formulation as the HBV ASO compound, or may be administered in a separate formulation. The one or more HBV therapeutic agents may be administered simultaneously with the ASO HBV compound, or may be administered separately, so that a dose of each of the HBV ASO compound and the HBV therapeutic agent overlap, in time, within the patient's body. In related embodiments, the one or more HBV therapeutic agent may be selected from interferon alpha-2b, interferon alpha-2a, and interferon alphacon-1 (pegylated and unpegylated), ribavirin; an HBV RNA replication inhibitor; a second HBV antisense compound; an HBV therapeutic vaccine; an HBV prophylactic vaccine; lamivudine (3TC); entecavir; tenofovir; telbivudine (LdT); adefovir; and an HBV antibody therapy (monoclonal or polyclonal).

EXAMPLES

Non-limiting disclosure and incorporation by reference

While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds described herein and are not intended to limit the same. Each of the references recited in the present application is incorporated herein by reference in its entirety.

Example 1: Antisense inhibition of HBV viral mRNA in HepG2.2.15 cells by MOE gapmers

Antisense oligonucleotides were designed targeting a HBV viral nucleic acid and were tested for their effects on HBV mRNA in vitro. Cultured HepG2.2.15 cells at a density of 25,000 cells per well were

transfected using electroporation with 15,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe set RTS3370 (forward sequence CTTGGTCATGGGCCATCAG, designated herein as SEQ ID NO: 2; reverse sequence CGGCTAGGAGTTCCGCAGTA, designated herein as SEQ ID NO: 3; probe sequence TGCGTGGAACCTTTTCGGCTCC, designated herein as SEQ ID NO: 4) was used to measure mRNA levels. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in Table 1 were designed as either 5-10-5 MOE gapmers or 3-10-4 MOE gapmers. The 5-10-5 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleosides each. The 3-10-4 MOE gapmers are 17 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising three and 4 nucleosides respectively. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has an MOE sugar modification. Each nucleoside in the central gap segment has a deoxy sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines.

"Viral Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the viral gene sequence. "Viral Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted viral gene sequence. The 'Motif' column indicates the gap and wing structure of each gapmer. Each gapmer listed in Table 1 is targeted to the viral genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. U95551.1).

Table 1

Inhibition of viral HBV mRNA levels by MOE gapmers targeted to SEQ ID NO: 1

Viral Start Site	Viral Stop Site	ISIS No	Sequence	Motif	% inhibition	SEQ ID NO
245	261	510088	CCACGAGTCTAGACTCT	3-10-4	55	5
250	266	510089	GTCCACCACGAGTCTAG	3-10-4	59	6
251	267	510090	AGTCCACCACGAGTCTA	3-10-4	60	7
252	268	510091	AAGTCCACCACGAGTCT	3-10-4	47	8
253	269	510092	GAAGTCCACCACGAGTC	3-10-4	59	9
254	270	510093	AGAAGTCCACCACGAGT	3-10-4	32	10
255	271	510094	GAGAAGTCCACCACGAG	3-10-4	41	11
256	272	510095	AGAGAAGTCCACCACGA	3-10-4	44	12
257	273	510096	GAGAGAAGTCCACCACG	3-10-4	54	13

258	274	510097	TGAGAGAAGTCCACCAC	3-10-4	57	14
384	400	510098	TGATAAAACGCCGCAGA	3-10-4	55	15
385	401	510099	ATGATAAAACGCCGCAG	3-10-4	59	16
411	427	510100	GGCATAGCAGCAGGATG	3-10-4	85	17
412	428	510101	AGGCATAGCAGCAGGAT	3-10-4	51	18
413	429	510102	GAGGCATAGCAGCAGGA	3-10-4	69	19
414	433	505330	AGATGAGGCATAGCAGCAGG	5-10-5	74	20
414	430	510103	TGAGGCATAGCAGCAGG	3-10-4	12	21
415	434	509928	AAGATGAGGCATAGCAGCAG	5-10-5	71	22
415	431	510104	ATGAGGCATAGCAGCAG	3-10-4	69	23
416	435	509929	GAAGATGAGGCATAGCAGCA	5-10-5	78	24
416	432	510105	GATGAGGCATAGCAGCA	3-10-4	69	25
417	436	509930	AGAAGATGAGGCATAGCAGC	5-10-5	72	26
417	433	510106	AGATGAGGCATAGCAGC	3-10-4	77	27
418	437	146783	AAGAAGATGAGGCATAGCAG	5-10-5	15	28
418	434	510107	AAGATGAGGCATAGCAG	3-10-4	69	29
419	435	510108	GAAGATGAGGCATAGCA	3-10-4	59	30
420	436	510109	AGAAGATGAGGCATAGC	3-10-4	0	31
421	437	510110	AAGAAGATGAGGCATAG	3-10-4	38	32
457	473	510111	ACGGGCAACATACCTTG	3-10-4	62	33
639	658	146784	CTGAGGCCCACTCCCATAGG	5-10-5	5	34
639	655	510112	AGGCCCACTCCCATAGG	3-10-4	44	35
640	656	510113	GAGGCCCACTCCCATAG	3-10-4	27	36
641	657	510114	TGAGGCCCACTCCCATA	3-10-4	44	37
642	658	510115	CTGAGGCCCACTCCCAT	3-10-4	52	38
687	706	509931	CGAACCACTGAACAAATGGC	5-10-5	89	39
687	703	510116	ACCACTGAACAAATGGC	3-10-4	89	40
688	704	510117	AACCACTGAACAAATGG	3-10-4	69	41
689	705	510118	GAACCACTGAACAAATG	3-10-4	63	42
690	706	510119	CGAACCACTGAACAAAT	3-10-4	74	43
738	754	510120	ACCACATCATCCATATA	3-10-4	71	44
1176	1192	510121	TCAGCAAACACTTGGCA	3-10-4	73	45
1778	1797	509932	AATTTATGCCTACAGCCTCC	5-10-5	76	46
1778	1794	510122	TTATGCCTACAGCCTCC	3-10-4	76	47
1779	1798	509933	CAATTTATGCCTACAGCCTC	5-10-5	72	48
1779	1795	510123	TTTATGCCTACAGCCTC	3-10-4	75	49
1780	1799	509934	CCAATTTATGCCTACAGCCT	5-10-5	75	50
1780	1796	510124	ATTTATGCCTACAGCCT	3-10-4	73	51
1781	1800	509935	ACCAATTTATGCCTACAGCC	5-10-5	72	52
1781	1797	510125	AATTTATGCCTACAGCC	3-10-4	69	53
1782	1798	510126	CAATTTATGCCTACAGC	3-10-4	59	54
1783	1799	510127	CCAATTTATGCCTACAG	3-10-4	58	55
1784	1800	510128	ACCAATTTATGCCTACA	3-10-4	60	56

1822	1838	510129	AGGCAGAGGTGAAAAAG	3-10-4	47	57
1823	1839	510130	TAGGCAGAGGTGAAAAA	3-10-4	30	58
1865	1884	509936	GCACAGCTTGGAGGCTTGAA	5-10-5	39	59
1865	1881	510131	CAGCTTGGAGGCTTGAA	3-10-4	4	60
1866	1885	509937	GGCACAGCTTGGAGGCTTGA	5-10-5	35	61
1866	1882	510132	ACAGCTTGGAGGCTTGA	3-10-4	0	62
1867	1886	505370	AGGCACAGCTTGGAGGCTTG	5-10-5	36	63
1867	1883	510133	CACAGCTTGGAGGCTTG	3-10-4	12	64
1868	1887	509938	AAGGCACAGCTTGGAGGCTT	5-10-5	7	65
1868	1884	510134	GCACAGCTTGGAGGCTT	3-10-4	20	66
1869	1888	509939	CAAGGCACAGCTTGGAGGCT	5-10-5	36	67
1869	1885	510135	GGCACAGCTTGGAGGCT	3-10-4	22	68
1870	1889	505371	CCAAGGCACAGCTTGGAGGC	5-10-5	35	69
1870	1886	510136	AGGCACAGCTTGGAGGC	3-10-4	14	70
1871	1887	510137	AAGGCACAGCTTGGAGG	3-10-4	0	71
1872	1888	510138	CAAGGCACAGCTTGGAG	3-10-4	6	72
1873	1889	510139	CCAAGGCACAGCTTGGA	3-10-4	17	73
1918	1934	510140	GCTCCAAATTCTTTATA	3-10-4	59	74
2378	2397	509940	TCTGCGAGGCGAGGGAGTTC	3-10-4	10	75
2378	2394	510141	GCGAGGCGAGGGAGTTC	3-10-4	5	76
2379	2395	510142	TGCGAGGCGAGGGAGTT	3-10-4	0	77
2380	2396	510143	CTGCGAGGCGAGGGAGT	3-10-4	8	78
2381	2397	510144	TCTGCGAGGCGAGGGAG	3-10-4	17	79
2820	2836	510145	TTCCCAAGAATATGGTG	3-10-4	22	80
2821	2837	510146	GTTCCCAAGAATATGGT	3-10-4	11	81
2822	2838	510147	TGTTCCCAAGAATATGG	3-10-4	21	82

Example 2: Antisense inhibition of HBV viral mRNA in HepG2.2.15 cells by MOE gapmers

Additional antisense oligonucleotides were designed targeting a HBV viral nucleic acid and were tested for their effects on HBV mRNA in vitro. Cultured HepG2.2.15 cells at a density of 25,000 cells per well were transfected using electroporation with 15,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe set RTS3370 was used to measure mRNA levels. RTS3370 detects the full length mRNA and the second portions of the pre-S1, pre-S2 and pre-C mRNA transcripts. The gapmers were also probed with additional primer probe sets. Viral primer probe set RTS3371 (forward sequence CCAAACCTTCGGACGGAAA, designated herein as SEQ ID NO: 311; reverse sequence TGAGGCCCACTCCCATAGG, designated herein as SEQ ID NO: 312; probe sequence CCCATCATCCTGGGCTTTCGGAAAAT, designated herein as SEQ ID NO: 313) was used also to measure mRNA levels. RTS3371 detects the full length mRNA and the second portions of the pre-S1, pre-S2 and pre-C mRNA transcripts, similar to RTS3370, but at different regions. Viral primer probe set RTS3372 (forward

sequence ATCCTATCAACACTTCCGGAAACT, designated herein as SEQ ID NO: 314; reverse sequence CGACGCGGCGATTGAG, designated herein as SEQ ID NO: 315; probe sequence AAGAACTCCCTCGCCTCGCAGACG, designated herein as SEQ ID NO: 316) was used to measure mRNA levels. RTS3372 detects the full length genomic sequence. Viral primer probe set RTS3373MGB (forward sequence CCGACCTTGAGGCATACTTCA, designated herein as SEQ ID NO: 317; reverse sequence AATTTATGCCTACAGCCTCCTAGTACA, designated herein as SEQ ID NO: 318; probe sequence TTAAAGACTGGGAGGAGTTG, designated herein as SEQ ID NO: 319) was used to measure mRNA levels. RTS3373MGB detects the full length mRNA and the second portions of the pre-S1, pre-S2, pre-C, and pre-X mRNA transcripts.

HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in Table 2 were designed as either 5-10-5 MOE gapmers, 3-10-3 MOE gapmers, or 2-10-2 MOE gapmers. The 5-10-5 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleosides each. The 3-10-3 MOE gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising three nucleosides each. The 2-10-2 MOE gapmers are 14 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising two nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has an MOE sugar modification. Each nucleoside in the central gap segment has a deoxy sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5'-methylcytosines.

"Start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the viral gene sequence. "Stop site" indicates the 3'-most nucleotide to which the gapmer is targeted viral gene sequence. The 'Motif' column indicates the gap and wing structure of each gapmer. Each gapmer listed in Table 2 is targeted to the viral genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. U95551.1).

Table 2

Inhibition of viral HBV mRNA levels by MOE gapmers targeted to SEQ ID NO: 1 (detected by RTS3370, RTS3371, RTS3372, and RTS3373MGB)

Start Site	Stop Site	ISIS No	Sequence	RTS3370 % inhibition	RTS3371 % inhibition	RTS3372 % inhibition	RTS3373 MGB % inhibition	Motif	SEQ ID NO
58	77	146779	GAACTGGA GCCACCAG CAGG	76	80	82	81	5-10-5	83
58	71	510019	GAGCCACC AGCAGG	38	32	45	31	2-10-2	84
61	80	505314	CCTGAACT GGAGCCAC CAGC	68	71	67	66	5-10-5	85
62	77	509941	GAACTGGA GCCACCAG	36	32	71	53	3-10-3	86
196	215	505315	AAAAACCC CGCCTGTA ACAC	69	74	80	88	5-10-5	87
199	218	505316	AAGAAAAA CCCCGCCTG TAA	60	60	64	64	5-10-5	88
205	224	505317	GTCAACAA GAAAAACC CCGC	85	83	79	85	5-10-5	89
228	241	510020	GTATTGTGA GGATT	28	18	0	16	2-10-2	90
229	242	510021	GGTATTGTG AGGAT	40	37	19	34	2-10-2	91
244	263	146821	CACCACGA GTCTAGACT CTG	74	73	62	75	5-10-5	92
245	260	509942	CACGAGTC TAGACTCT	18	15	45	46	3-10-3	93
245	258	510022	CGAGTCTA GACTCT	32	26	23	19	2-10-2	94
246	261	509943	CCACGAGT CTAGACTC	34	35	63	60	3-10-3	95
247	266	505318	GTCCACCA CGAGTCTA GACT	75	77	64	75	5-10-5	96
250	269	509921	GAAGTCCA CCACGAGT CTAG	46	46	39	40	5-10-5	97
250	265	509944	TCCACCAC GAGTCTAG	38	39	65	59	3-10-3	98
251	270	509922	AGAAGTCC ACCACGAG TCTA	55	56	17	38	5-10-5	99

251	266	509945	GTCCACCA CGAGTCTA	34	35	64	51	3-10-3	100
252	271	509923	GAGAAGTC CACCACGA GTCT	39	38	39	33	5-10-5	101
252	267	509946	AGTCCACC ACGAGTCT	47	51	50	45	3-10-3	102
253	272	505319	AGAGAAGT CCACCACG AGTC	88	83	80	78	5-10-5	103
253	268	509947	AAGTCCAC CACGAGTC	46	50	56	46	3-10-3	104
254	273	509924	GAGAGAAG TCCACCAC GAGT	43	40	49	44	5-10-5	105
254	269	509948	GAAGTCCA CCACGAGT	41	46	51	44	3-10-3	106
254	267	510023	AGTCCACC ACGAGT	41	32	47	48	2-10-2	107
255	274	509925	TGAGAGAA GTCCACCA CGAG	50	57	55	55	5-10-5	108
255	270	509949	AGAAGTCC ACCACGAG	40	41	52	34	3-10-3	109
255	268	510024	AAGTCCAC CACGAG	26	29	19	23	2-10-2	110
256	275	505320	TTGAGAGA AGTCCACC ACGA	51	57	55	66	5-10-5	111
256	271	509950	GAGAAGTC CACCACGA	30	31	43	33	3-10-3	112
256	269	510025	GAAGTCCA CCACGA	44	38	53	54	2-10-2	113
257	270	510026	AGAAGTCC ACCACG	39	42	32	25	2-10-2	114
258	273	509952	GAGAGAAG TCCACCAC	54	52	60	48	3-10-3	115
258	271	510027	GAGAAGTC CACCAC	29	30	25	19	2-10-2	116
259	274	509953	TGAGAGAA GTCCACCA	39	44	47	38	3-10-3	117
259	272	510028	AGAGAAGT CCACCA	31	29	3	15	2-10-2	118
260	273	510029	GAGAGAAG TCCACC	21	19	23	18	2-10-2	119
261	274	510030	TGAGAGAA GTCCAC	16	22	21	20	2-10-2	120
262	281	505321	AGAAAATT GAGAGAAG TCCA	53	58	52	56	5-10-5	121

265	284	505322	CCTAGAAA ATTGAGAG AAGT	62	65	69	67	5-10-5	122
293	312	505323	ATTTTGGCC AAGACACA CGG	86	84	81	85	5-10-5	123
296	315	505324	CGAATTTTG GCCAAGAC ACA	67	67	69	64	5-10-5	124
302	321	505325	GGACTGCG AATTTTGGC CAA	77	75	73	76	5-10-5	125
360	379	505326	TCCAGCGA TAACCAGG ACAA	89	90	77	91	5-10-5	126
366	385	505327	GACACATC CAGCGATA ACCA	83	85	75	86	5-10-5	127
369	388	505328	GCAGACAC ATCCAGCG ATAA	65	68	49	57	5-10-5	128
384	399	509954	GATAAAAC GCCGCAGA	37	46	53	35	3-10-3	129
384	397	510031	TAAAACGC CGCAGA	36	36	33	33	2-10-2	130
385	398	510032	ATAAAACG CCGCAG	12	7	19	15	2-10-2	131
386	401	509955	ATGATAAA ACGCCGCA	49	55	57	53	3-10-3	132
386	399	510033	GATAAAAC GCCGCA	39	39	45	37	2-10-2	133
387	400	510034	TGATAAAA CGCCGC	40	37	29	39	2-10-2	134
388	401	510035	ATGATAAA ACGCCG	22	24	9	22	2-10-2	135
411	430	505329	TGAGGCAT AGCAGCAG GATG	60	64	47	55	5-10-5	136
411	426	509956	GCATAGCA GCAGGATG	62	64	71	60	3-10-3	137
411	424	510036	ATAGCAGC AGGATG	44	34	30	48	2-10-2	138
412	431	509926	ATGAGGCA TAGCAGCA GGAT	45	54	71	62	5-10-5	139
412	427	509957	GGCATAGC AGCAGGAT	72	75	80	71	3-10-3	140
412	425	510037	CATAGCAG CAGGAT	29	24	24	20	2-10-2	141
413	432	509927	GATGAGGC ATAGCAGC AGGA	54	58	54	49	5-10-5	142

413	428	509958	AGGCATAG CAGCAGGA	63	66	68	64	3-10-3	143
413	426	510038	GCATAGCA GCAGGA	55	54	37	46	2-10-2	144
414	433	505330	AGATGAGG CATAGCAG CAGG	85	87	74	82	5-10-5	20
414	429	509959	GAGGCATA GCAGCAGG	64	64	80	68	3-10-3	145
414	427	510039	GGCATAGC AGCAGG	58	54	41	45	2-10-2	146
415	430	509960	TGAGGCAT AGCAGCAG	59	59	66	64	3-10-3	147
415	428	510040	AGGCATAG CAGCAG	58	55	38	41	2-10-2	148
416	431	509961	ATGAGGCA TAGCAGCA	56	54	65	56	3-10-3	149
416	429	510041	GAGGCATA GCAGCA	64	62	64	57	2-10-2	150
417	432	509962	GATGAGGC ATAGCAGC	57	52	58	49	3-10-3	151
417	430	510042	TGAGGCAT AGCAGC	48	50	55	48	2-10-2	152
418	433	509963	AGATGAGG CATAGCAG	50	52	64	51	3-10-3	153
418	431	510043	ATGAGGCA TAGCAG	36	31	36	26	2-10-2	154
419	434	509964	AAGATGAG GCATAGCA	48	47	72	65	3-10-3	155
419	432	510044	GATGAGGC ATAGCA	44	28	0	14	2-10-2	156
420	435	509965	GAAGATGA GGCATAGC	45	41	65	62	3-10-3	157
420	433	510045	AGATGAGG CATAGC	41	43	37	29	2-10-2	158
421	436	509966	AGAAGATG AGGCATAG	32	29	64	51	3-10-3	159
421	434	510046	AAGATGAG GCATAG	21	18	26	27	2-10-2	160
422	437	509967	AAGAAGAT GAGGCATA	21	17	55	46	3-10-3	161
422	435	510047	GAAGATGA GGCATA	25	24	23	25	2-10-2	162
423	436	510048	AGAAGATG AGGCAT	21	17	25	19	2-10-2	163
424	437	510049	AAGAAGAT GAGGCA	17	11	38	27	2-10-2	164
454	473	505331	ACGGGCAA CATACCTTG ATA	55	57	65	60	5-10-5	165

457	476	505332	CAAACGGG CAACATAC CTTG	73	77	77	74	5-10-5	166
457	472	509968	CGGGCAAC ATACCTTG	60	61	73	70	3-10-3	167
458	473	509969	ACGGGCAA CATACCTT	58	63	64	58	3-10-3	168
458	471	510050	GGGCAACA TACCTT	58	56	57	46	2-10-2	169
459	472	510051	CGGGCAAC ATACCT	49	43	47	37	2-10-2	170
460	473	510052	ACGGGCAA CATACC	50	50	54	51	2-10-2	171
463	482	505333	AGAGGACA AACGGGCA ACAT	64	68	64	71	5-10-5	172
466	485	505334	ATTAGAGG ACAAACGG GCAA	59	62	42	69	5-10-5	173
472	491	505335	CCTGGAATT AGAGGACA AAC	78	81	73	86	5-10-5	174
475	494	505336	GATCCTGG AATTAGAG GACA	56	65	61	72	5-10-5	175
639	654	509970	GGCCCACT CCCATAGG	38	55	74	48	3-10-3	176
641	656	509971	GAGGCCCA CTCCCATA	30	46	77	54	3-10-3	177
642	657	509972	TGAGGCCC ACTCCCAT	58	57	84	66	3-10-3	178
643	658	509973	CTGAGGCC CACTCCCA	38	53	70	66	3-10-3	179
670	689	146823	GGCACTAG TAAACTGA GCCA	61	64	63	63	5-10-5	180
670	685	509974	CTAGTAAA CTGAGCCA	71	71	78	80	3-10-3	181
670	683	510053	AGTAAACT GAGCCA	49	48	52	53	2-10-2	182
671	684	510054	TAGTAAAC TGAGCC	41	38	19	30	2-10-2	183
672	685	510055	CTAGTAAA CTGAGC	25	27	42	47	2-10-2	184
673	692	505337	AATGGCAC TAGTAAAC TGAG	34	46	49	52	5-10-5	185
679	698	505338	TGAACAAA TGGCACTA GTAA	74	77	71	80	5-10-5	186

682	701	505339	CACTGAAC AAATGGCA CTAG	82	83	71	82	5-10-5	187
687	702	509975	CCACTGAA CAAATGGC	72	73	76	80	3-10-3	188
688	707	505340	ACGAACCA CTGAACAA ATGG	69	69	78	76	5-10-5	189
688	703	509976	ACCACTGA ACAAATGG	47	48	67	65	3-10-3	190
689	704	509977	AACCACTG AACAAATG	33	33	39	41	3-10-3	191
690	705	509978	GAACCACT GAACAAAT	50	49	63	48	3-10-3	192
691	710	505341	CCTACGAA CCACTGAA CAAA	64	70	70	72	5-10-5	193
691	706	509979	CGAACCAC TGAACAAA	67	66	78	77	3-10-3	194
691	704	510056	AACCACTG AACAAA	36	36	23	32	2-10-2	195
692	705	510057	GAACCACT GAACAA	45	44	51	43	2-10-2	196
693	706	510058	CGAACCAC TGAACA	59	52	48	49	2-10-2	197
697	716	505342	GAAAGCCC TACGAACC ACTG	76	80	73	83	5-10-5	198
738	753	509980	CCACATCAT CCATATA	40	33	62	54	3-10-3	199
738	751	510059	ACATCATCC ATATA	19	9	30	27	2-10-2	200
739	754	509981	ACCACATC ATCCATAT	76	78	93	85	3-10-3	201
739	752	510060	CACATCATC CATAT	45	35	24	17	2-10-2	202
740	753	510061	CCACATCAT CCATA	52	49	43	40	2-10-2	203
741	754	510062	ACCACATC ATCCAT	44	45	48	47	2-10-2	204
756	775	505343	TGTACAGA CTTGGCCCC CAA	47	56	55	68	5-10-5	205
823	842	505344	AGGGTTTA AATGTATA CCCA	66	71	64	72	5-10-5	206
1170	1189	505345	GCAAACAC TTGGCACA GACC	76	80	35	70	5-10-5	207
1176	1191	509982	CAGCAAAC ACTTGGCA	42	44	56	54	3-10-3	208

1177	1192	509983	TCAGCAAA CACTTGGC	60	54	74	70	3-10-3	209
1259	1278	505346	CCGCAGTA TGGATCGG CAGA	88	82	57	80	5-10-5	210
1261	1276	509984	GCAGTATG GATCGGCA	61	58	65	72	3-10-3	211
1262	1281	505347	GTTCCGCA GTATGGAT CGGC	84	81	71	83	5-10-5	212
1268	1287	505348	CTAGGAGT TCCGCAGT ATGG	78	68	70	79	5-10-5	213
1271	1290	505349	CGGCTAGG AGTTCCGC AGTA	47	54	59	61	5-10-5	214
1277	1296	505350	AACAAGCG GCTAGGAG TTCC	55	62	69	69	5-10-5	215
1280	1299	505351	CAAAACAA GCGGCTAG GAGT	20	49	49	54	5-10-5	216
1283	1302	505352	GAGCAAAA CAAGCGGC TAGG	53	83	73	87	5-10-5	217
1286	1305	505353	TGCGAGCA AAACAAGC GGCT	64	73	68	78	5-10-5	218
1413	1426	510063	ACAAAGGA CGTCCC	14	8	0	0	2-10-2	219
1515	1534	505354	GAGGTGCG CCCCGTGGT CGG	68	81	61	80	5-10-5	220
1518	1537	505355	AGAGAGGT GCGCCCCG TGGT	59	75	75	84	5-10-5	221
1521	1540	505356	TAAAGAGA GGTGCGCC CCGT	63	76	83	78	5-10-5	222
1550	1563	510064	AAGGCACA GACGGG	35	38	25	32	2-10-2	223
1577	1596	146786	GTGAAGCG AAGTGCAC ACGG	88	91	84	93	5-10-5	224
1580	1599	505357	GAGGTGAA GCGAAGTG CACA	70	75	71	82	5-10-5	225
1583	1602	505358	GCAGAGGT GAAGCGAA GTGC	77	82	72	84	5-10-5	226

1586	1605	505359	CGTGCAGA GGTGAAGC GAAG	72	73	67	80	5-10-5	227
1655	1674	505360	AGTCCAAG AGTCCTCTT ATG	66	68	54	68	5-10-5	228
1706	1719	510065	CAGTCTTTG AAGTA	19	19	26	17	2-10-2	229
1778	1793	509985	TATGCCTAC AGCCTCC	64	60	64	63	3-10-3	230
1779	1794	509986	TTATGCCTA CAGCCTC	66	66	77	73	3-10-3	231
1780	1795	509987	TTTATGCCT ACAGCCT	56	55	68	67	3-10-3	232
1781	1796	509988	ATTTATGCC TACAGCC	52	52	68	63	3-10-3	233
1782	1797	509989	AATTTATGC CTACAGC	48	44	70	59	3-10-3	234
1783	1798	509990	CAATTTATG CCTACAG	24	18	39	40	3-10-3	235
1784	1799	509991	CCAATTTAT GCCTACA	37	37	55	55	3-10-3	236
1785	1800	509992	ACCAATTTA TGCCTAC	35	36	60	55	3-10-3	237
1806	1825	505361	AAAGTTGC ATGGTGCT GGTG	42	55	75	61	5-10-5	238
1809	1828	505362	GAAAAAGT TGCATGGT GCTG	45	56	64	53	5-10-5	239
1812	1831	505363	GGTGAAAA AGTTGCAT GGTG	71	70	80	72	5-10-5	240
1815	1834	505364	AGAGGTGA AAAAGTTG CATG	51	57	77	82	5-10-5	241
1818	1837	505365	GGCAGAGG TGAAAAAG TTGC	54	63	76	78	5-10-5	242
1821	1840	505366	TTAGGCAG AGGTGAAA AAGT	61	65	80	66	5-10-5	243
1822	1837	509993	GGCAGAGG TGAAAAAG	47	51	74	54	3-10-3	244
1823	1838	509994	AGGCAGAG GTGAAAAA	47	40	76	54	3-10-3	245
1824	1843	505367	TGATTAGG CAGAGGTG AAAA	41	39	62	29	5-10-5	246
1824	1839	509995	TAGGCAGA GGTGAAAA	46	42	79	59	3-10-3	247

1826	1839	510066	TAGGCAGA GGTGAA	40	33	44	31	2-10-2	248
1827	1846	505368	AGATGATT AGGCAGAG GTGA	27	46	62	51	5-10-5	249
1861	1880	146787	AGCTTGGA GGCTTGAA CAGT	59	61	65	72	5-10-5	250
1864	1883	505369	CACAGCTT GGAGGCTT GAAC	11	21	48	31	5-10-5	251
1865	1880	509996	AGCTTGGA GGCTTGAA	13	1	45	40	3-10-3	252
1865	1878	510067	CTTGGAGG CTTGAA	22	17	20	14	2-10-2	253
1866	1881	509997	CAGCTTGG AGGCTTGA	29	19	51	45	3-10-3	254
1866	1879	510068	GCTTGGAG GCTTGA	24	25	37	32	2-10-2	255
1867	1886	505370	AGGCACAG CTTGGAGG CTTG	32	36	58	33	5-10-5	63
1867	1882	509998	ACAGCTTG GAGGCTTG	1	4	23	12	3-10-3	256
1867	1880	510069	AGCTTGGA GGCTTG	23	24	17	23	2-10-2	257
1868	1883	509999	CACAGCTT GGAGGCTT	5	1	48	41	3-10-3	258
1868	1881	510070	CAGCTTGG AGGCTT	21	20	0	18	2-10-2	259
1869	1884	510000	GCACAGCT TGGAGGCT	14	10	50	37	3-10-3	260
1869	1882	510071	ACAGCTTG GAGGCT	19	22	24	27	2-10-2	261
1870	1889	505371	CCAAGGCA CAGCTTGG AGGC	27	40	68	38	5-10-5	69
1870	1885	510001	GGCACAGC TTGGAGGC	10	12	43	16	3-10-3	262
1870	1883	510072	CACAGCTT GGAGGC	28	31	33	30	2-10-2	263
1871	1886	510002	AGGCACAG CTTGGAGG	24	20	46	25	3-10-3	264
1871	1884	510073	GCACAGCT TGGAGG	20	18	22	15	2-10-2	265
1872	1887	510003	AAGGCACA GCTTGGAG	6	0	45	24	3-10-3	266
1872	1885	510074	GGCACAGC TTGGAG	18	18	32	23	2-10-2	267

1873	1892	505372	CACCCAAG GCACAGCT TGGA	18	8	55	16	5-10-5	268
1873	1888	510004	CAAGGCAC AGCTTGGA	9	0	31	15	3-10-3	269
1873	1886	510075	AGGCACAG CTTGGA	23	9	27	10	2-10-2	270
1874	1889	510005	CCAAGGCA CAGCTTGG	0	0	39	25	3-10-3	271
1876	1895	505373	AGCCACCC AAGGCACA GCTT	47	50	69	56	5-10-5	272
1879	1898	505374	CAAAGCCA CCCAAGGC ACAG	27	27	55	30	5-10-5	273
1882	1901	505375	CCCCAAAG CCACCCAA GGCA	34	40	54	39	5-10-5	274
1885	1904	505376	ATGCCCCA AAGCCACC CAAG	41	43	54	52	5-10-5	275
1888	1907	505377	TCCATGCCC CAAAGCCA CCC	40	42	72	40	5-10-5	276
1891	1910	505378	ATGTCCATG CCCCAAAG CCA	35	33	70	40	5-10-5	277
1918	1933	510006	CTCCAAATT CTTTATA	9	2	53	41	3-10-3	278
1918	1931	510076	CCAAATTCT TTATA	28	22	7	22	2-10-2	279
1919	1934	510007	GCTCCAAA TTCTTTAT	43	39	72	57	3-10-3	280
1919	1932	510077	TCCAAATTC TTTAT	19	11	0	2	2-10-2	281
1920	1933	510078	CTCCAAATT CTTTA	19	11	0	0	2-10-2	282
1921	1934	510079	GCTCCAAA TTCTTT	50	48	61	55	2-10-2	283
1957	1976	505379	GGAAAGAA GTCAGAAG GCAA	17	14	81	39	5-10-5	284
2270	2285	510008	GTGCGAAT CCACACTC	21	4	36	11	3-10-3	285
2270	2283	510080	GCGAATCC ACACTC	32	29	41	33	2-10-2	286
2271	2284	510081	TGCGAATC CACACT	28	20	25	11	2-10-2	287
2272	2285	510082	GTGCGAAT CCACAC	28	20	32	22	2-10-2	288

2368	2387	505380	GAGGGAGT TCTTCTTCT AGG	24	22	90	48	5-10-5	289
2378	2393	510009	CGAGGCGA GGGAGTTC	12	1	65	10	3-10-3	290
2378	2391	510083	AGGCGAGG GAGTTC	17	18	29	25	2-10-2	291
2379	2394	510010	GCGAGGCG AGGGAGTT	18	13	82	37	3-10-3	292
2379	2392	510084	GAGGCGAG GGAGTT	29	22	54	30	2-10-2	293
2380	2395	510011	TGCGAGGC GAGGGAGT	13	11	69	44	3-10-3	294
2380	2393	510085	CGAGGCGA GGGAGT	25	20	53	42	2-10-2	295
2381	2396	510012	CTGCGAGG CGAGGGAG	17	14	79	53	3-10-3	296
2381	2394	510086	GCGAGGCG AGGGAG	33	29	66	48	2-10-2	297
2382	2397	510013	TCTGCGAG GCGAGGGA	18	4	77	47	3-10-3	298
2420	2439	505381	CCGAGATT GAGATCTTC TGC	12	18	83	28	5-10-5	299
2459	2478	505382	CCCACCTTA TGAGTCCA AGG	14	19	80	36	5-10-5	300
2819	2838	505383	TGTTCCCAA GAATATGG TGA	29	32	78	44	5-10-5	301
2820	2835	510014	TCCCAAGA ATATGGTG	10	10	68	40	3-10-3	302
2821	2836	510015	TTCCCAAG AATATGGT	5	0	62	24	3-10-3	303
2822	2837	510016	GTTCCCAA GAATATGG	6	2	42	16	3-10-3	304
2823	2838	510017	TGTTCCCAA GAATATG	18	18	47	18	3-10-3	305
2824	2839	510018	TTGTTCCCA AGAATAT	7	5	57	19	3-10-3	306
2825	2838	510087	TGTTCCCAA GAATA	25	20	44	25	2-10-2	307
2873	2892	505384	GAAAGAAT CCCAGAGG ATTG	8	4	61	22	5-10-5	308
3161	3180	146833	ACTGCATG GCCTGAGG ATGA	47	46	82	54	5-10-5	309
3163	3182	505385	CCACTGCAT GGCCTGAG GAT	25	34	69	19	5-10-5	310

Example 3: Antisense inhibition of HBV viral mRNA in HepAD38 (Tet-HBV) cells by MOE gapmers

Certain antisense oligonucleotides selected from the study described in Example 2 were tested for their effects on HBV mRNA in another cell line, human hepatoma HepAD38 cells, in which HBV production is under the control of a tetracycline-regulated promoter. Cultured HepAD38 (Tet-HBV) cells at a density of 45,000 cells per well were transfected using electroporation with 15,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe sets RTS3372 and RTS3373MGB were used individually to measure mRNA levels. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented in Table 3 as percent inhibition of HBV, relative to untreated control cells.

Table 3

Inhibition of viral HBV mRNA levels by MOE gapmersin HepAD38 (Tet-HBV) cells (detected by RTS3372 and RTS3373MGB)

Start Site	Stop Site	ISIS No	Motif	RTS3373MGB % inhibition	RTS3372 % inhibition	SEQ ID NO
58	77	146779	5-10-5	76	82	83
58	71	510019	5-10-5	0	9	84
61	80	505314	5-10-5	65	75	85
196	215	505315	5-10-5	46	65	87
199	218	505316	5-10-5	57	71	88
205	224	505317	5-10-5	83	87	89
228	241	510020	2-10-2	6	0	90
229	242	510021	2-10-2	19	24	91
244	263	146821	5-10-5	72	71	92
245	258	510022	2-10-2	6	24	94
247	266	505318	5-10-5	68	77	96
250	269	509921	5-10-5	25	47	97
251	270	509922	5-10-5	28	46	99
252	271	509923	5-10-5	19	40	101
253	272	505319	5-10-5	69	66	103
254	273	509924	5-10-5	9	39	105
254	267	510023	2-10-2	19	15	107
255	274	509925	5-10-5	26	55	108
255	268	510024	2-10-2	0	5	110
256	275	505320	5-10-5	62	68	111
256	269	510025	2-10-2	0	8	113
257	270	510026	2-10-2	7	21	114
258	271	510027	2-10-2	0	0	116
259	272	510028	2-10-2	0	0	118
260	273	510029	2-10-2	0	9	119

261	274	510030	2-10-2	0	0	120
262	281	505321	5-10-5	53	54	121
265	284	505322	5-10-5	59	60	122
293	312	505323	5-10-5	65	77	123
296	315	505324	5-10-5	78	83	124
302	321	505325	5-10-5	71	80	125
360	379	505326	5-10-5	76	84	126
366	385	505327	5-10-5	77	83	127
369	388	505328	5-10-5	65	78	128
384	397	510031	2-10-2	0	16	130
385	398	510032	2-10-2	0	0	131
386	399	510033	2-10-2	1	21	133
387	400	510034	2-10-2	8	28	134
388	401	510035	2-10-2	0	0	135
411	430	505329	5-10-5	58	72	136
411	424	510036	2-10-2	6	11	138
412	431	509926	5-10-5	20	54	139
412	425	510037	2-10-2	0	10	141
413	432	509927	5-10-5	56	76	142
413	426	510038	2-10-2	54	68	144
414	433	505330	5-10-5	66	81	20
414	427	510039	2-10-2	60	74	146
415	428	510040	2-10-2	33	39	148
416	429	510041	2-10-2	30	58	150
417	430	510042	2-10-2	34	57	152
418	431	510043	2-10-2	0	2	154
419	432	510044	2-10-2	0	29	156
420	433	510045	2-10-2	3	31	158
421	434	510046	2-10-2	0	0	160
422	435	510047	2-10-2	0	0	162
423	436	510048	2-10-2	0	0	163
424	437	510049	2-10-2	0	0	164
454	473	505331	5-10-5	60	77	165
457	476	505332	5-10-5	55	74	166
458	471	510050	2-10-2	47	47	169
459	472	510051	2-10-2	35	55	170
460	473	510052	2-10-2	27	41	171
463	482	505333	5-10-5	66	78	172
466	485	505334	5-10-5	53	63	173
472	491	505335	5-10-5	70	76	174
475	494	505336	5-10-5	64	77	175
670	689	146823	5-10-5	74	79	180
670	683	510053	2-10-2	18	20	182

671	684	510054	2-10-2	13	21	183
672	685	510055	2-10-2	4	2	184
673	692	505337	5-10-5	60	72	185
679	698	505338	5-10-5	62	75	186
682	701	505339	5-10-5	81	90	187
688	707	505340	5-10-5	67	81	189
691	710	505341	5-10-5	68	80	193
691	704	510056	2-10-2	0	0	195
692	705	510057	2-10-2	37	48	196
693	706	510058	2-10-2	44	59	197
697	716	505342	5-10-5	80	87	198
738	751	510059	2-10-2	0	0	200
739	752	510060	2-10-2	0	0	202
740	753	510061	2-10-2	23	19	203
741	754	510062	2-10-2	25	30	204
756	775	505343	5-10-5	62	71	205
823	842	505344	5-10-5	52	66	206
1170	1189	505345	5-10-5	83	81	207
1259	1278	505346	5-10-5	84	81	210
1262	1281	505347	5-10-5	89	84	212
1268	1287	505348	5-10-5	78	78	213
1271	1290	505349	5-10-5	74	77	214
1277	1296	505350	5-10-5	75	77	215
1280	1299	505351	5-10-5	49	62	216
1283	1302	505352	5-10-5	70	66	217
1286	1305	505353	5-10-5	62	60	218
1413	1426	510063	2-10-2	0	0	219
1515	1534	505354	5-10-5	85	75	220
1518	1537	505355	5-10-5	81	74	221
1521	1540	505356	5-10-5	57	52	222
1550	1563	510064	2-10-2	0	0	223
1577	1596	146786	5-10-5	94	85	224
1580	1599	505357	5-10-5	86	79	225
1583	1602	505358	5-10-5	89	79	226
1586	1605	505359	5-10-5	82	68	227
1655	1674	505360	5-10-5	84	74	228
1706	1719	510065	2-10-2	0	0	229
1806	1825	505361	5-10-5	66	66	238
1809	1828	505362	5-10-5	52	59	239
1812	1831	505363	5-10-5	72	75	240
1815	1834	505364	5-10-5	73	80	241
1818	1837	505365	5-10-5	68	82	242
1821	1840	505366	5-10-5	50	76	243

1824	1843	505367	5-10-5	58	76	246
1826	1839	510066	2-10-2	0	31	248
1827	1846	505368	5-10-5	71	84	249
1861	1880	146787	5-10-5	25	35	250
1864	1883	505369	5-10-5	29	65	251
1865	1878	510067	2-10-2	0	0	253
1866	1879	510068	2-10-2	0	20	255
1867	1886	505370	5-10-5	45	70	63
1867	1880	510069	2-10-2	0	0	257
1868	1881	510070	2-10-2	0	0	259
1869	1882	510071	2-10-2	0	0	261
1870	1889	505371	5-10-5	48	66	69
1870	1883	510072	2-10-2	0	0	263
1871	1884	510073	2-10-2	0	0	265
1872	1885	510074	2-10-2	0	2	267
1873	1892	505372	5-10-5	48	67	268
1873	1886	510075	2-10-2	0	0	270
1876	1895	505373	5-10-5	23	48	272
1879	1898	505374	5-10-5	0	34	273
1882	1901	505375	5-10-5	39	66	274
1885	1904	505376	5-10-5	0	40	275
1888	1907	505377	5-10-5	4	47	276
1891	1910	505378	5-10-5	65	77	277
1918	1931	510076	2-10-2	0	0	279
1919	1932	510077	2-10-2	0	0	281
1920	1933	510078	2-10-2	0	0	282
1921	1934	510079	2-10-2	18	50	283
1957	1976	505379	5-10-5	42	84	284
2270	2283	510080	2-10-2	0	0	286
2271	2284	510081	2-10-2	0	0	287
2272	2285	510082	2-10-2	0	10	288
2368	2387	505380	5-10-5	29	79	289
2378	2391	510083	2-10-2	0	0	291
2379	2392	510084	2-10-2	31	17	293
2380	2393	510085	2-10-2	0	8	295
2381	2394	510086	2-10-2	10	2	297
2420	2439	505381	5-10-5	30	86	299
2459	2478	505382	5-10-5	16	87	300
2819	2838	505383	5-10-5	26	81	301
2825	2838	510087	2-10-2	0	0	307
2873	2892	505384	5-10-5	31	59	308
3161	3180	146833	5-10-5	55	76	309
3163	3182	505385	5-10-5	58	83	310

Example 4: Antisense inhibition of HBV viral mRNA in HepAD38 (Tet-HBV) cells by MOE gapmers

Certain antisense oligonucleotides from the study described in Examples 1 and 2 were tested for their effects on HBV mRNA *in vitro*. Cultured HepAD38 (Tet-HBV) cells at a density of 45,000 cells per well were transfected using electroporation with 15,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe set RTS3372 was used to measure mRNA levels. The mRNA levels were also measured using the RTS3373MGB primer probe set. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented in Table 4 as percent inhibition of HBV, relative to untreated control cells.

Table 4

Inhibition of viral HBV mRNA levels by MOE gapmers (RTS3372 and RTS3373MGB)

Start Site	Stop Site	ISIS No	Motif	RTS3372 % inhibition	RTS3373MGB % inhibition	SEQ ID NO
62	77	509941	3-10-3	36	5	86
245	260	509942	3-10-3	3	0	93
245	261	510088	3-10-4	24	10	5
246	261	509943	3-10-3	27	13	95
250	265	509944	3-10-3	46	34	98
250	266	510089	3-10-4	61	33	6
251	266	509945	3-10-3	54	43	100
251	267	510090	3-10-4	58	32	7
252	267	509946	3-10-3	50	28	102
252	268	510091	3-10-4	60	42	8
253	268	509947	3-10-3	49	40	104
253	269	510092	3-10-4	40	9	9
254	269	509948	3-10-3	13	22	106
254	270	510093	3-10-4	39	2	10
255	270	509949	3-10-3	33	24	109
255	271	510094	3-10-4	40	16	11
256	271	509950	3-10-3	31	23	112
256	272	510095	3-10-4	24	6	12
257	273	510096	3-10-4	62	44	13
258	273	509952	3-10-3	42	40	115
258	274	510097	3-10-4	65	48	14
259	274	509953	3-10-3	35	29	117
384	399	509954	3-10-3	35	18	129
384	400	510098	3-10-4	62	43	15
385	401	510099	3-10-4	67	50	16
386	401	509955	3-10-3	44	37	132

411	426	509956	3-10-3	67	53	137
411	427	510100	3-10-4	88	69	17
412	427	509957	3-10-3	86	76	140
412	428	510101	3-10-4	71	46	18
413	428	509958	3-10-3	78	74	143
413	429	510102	3-10-4	77	52	19
414	433	505330	5-10-5	81	60	20
414	429	509959	3-10-3	62	49	145
414	430	510103	3-10-4	9	5	21
415	434	509928	5-10-5	81	66	22
415	430	509960	3-10-3	67	57	147
415	431	510104	3-10-4	71	57	23
416	435	509929	5-10-5	82	69	24
416	431	509961	3-10-3	62	43	149
416	432	510105	3-10-4	81	64	25
417	436	509930	5-10-5	74	45	26
417	432	509962	3-10-3	59	48	151
417	433	510106	3-10-4	86	70	27
418	437	146783	5-10-5	19	3	28
418	433	509963	3-10-3	48	28	153
418	434	510107	3-10-4	74	51	29
419	434	509964	3-10-3	50	39	155
419	435	510108	3-10-4	67	50	30
420	435	509965	3-10-3	49	38	157
420	436	510109	3-10-4	12	13	31
421	436	509966	3-10-3	23	22	159
421	437	510110	3-10-4	34	16	32
422	437	509967	3-10-3	3	12	161
457	472	509968	3-10-3	56	38	167
457	473	510111	3-10-4	68	51	33
458	473	509969	3-10-3	53	39	168
639	658	146784	5-10-5	0	0	34
639	654	509970	3-10-3	51	15	176
639	655	510112	3-10-4	66	32	35
640	656	510113	3-10-4	70	31	36
641	656	509971	3-10-3	54	31	177
641	657	510114	3-10-4	67	45	37
642	657	509972	3-10-3	51	25	178
642	658	510115	3-10-4	73	50	38
643	658	509973	3-10-3	49	32	179
670	685	509974	3-10-3	74	67	181
687	706	509931	5-10-5	92	83	39
687	702	509975	3-10-3	72	71	188

687	703	510116	3-10-4	83	74	40
688	703	509976	3-10-3	46	52	190
688	704	510117	3-10-4	71	57	41
689	704	509977	3-10-3	18	22	191
689	705	510118	3-10-4	71	50	42
690	705	509978	3-10-3	57	37	192
690	706	510119	3-10-4	80	64	43
691	706	509979	3-10-3	65	55	194
738	753	509980	3-10-3	48	44	199
738	754	510120	3-10-4	70	54	44
739	754	509981	3-10-3	54	45	201
1176	1191	509982	3-10-3	44	36	208
1176	1192	510121	3-10-4	74	69	45
1177	1192	509983	3-10-3	57	53	209
1261	1276	509984	3-10-3	57	50	211
1778	1797	509932	5-10-5	30	76	46
1778	1793	509985	3-10-3	0	46	230
1778	1794	510122	3-10-4	0	60	47
1779	1798	509933	5-10-5	54	78	48
1779	1794	509986	3-10-3	56	81	231
1779	1795	510123	3-10-4	74	85	49
1780	1799	509934	5-10-5	69	84	50
1780	1795	509987	3-10-3	52	78	232
1780	1796	510124	3-10-4	75	84	51
1781	1800	509935	5-10-5	72	85	52
1781	1796	509988	3-10-3	57	68	232
1781	1797	510125	3-10-4	68	72	53
1782	1797	509989	3-10-3	46	41	234
1782	1798	510126	3-10-4	56	51	54
1783	1798	509990	3-10-3	16	25	234
1783	1799	510127	3-10-4	61	69	55
1784	1799	509991	3-10-3	41	41	236
1784	1800	510128	3-10-4	61	68	56
1785	1800	509992	3-10-3	43	43	237
1822	1837	509993	3-10-3	72	44	244
1822	1838	510129	3-10-4	66	33	57
1823	1838	509994	3-10-3	79	32	245
1823	1839	510130	3-10-4	49	31	58
1824	1839	509995	3-10-3	63	30	247
1865	1884	509936	5-10-5	74	59	59
1865	1880	509996	3-10-3	36	0	252
1865	1881	510131	3-10-4	26	0	60
1866	1885	509937	5-10-5	78	63	61

1866	1881	509997	3-10-3	5	0	254
1866	1882	510132	3-10-4	37	4	62
1867	1886	505370	5-10-5	54	17	63
1867	1882	509998	3-10-3	13	0	256
1867	1883	510133	3-10-4	42	25	64
1868	1887	509938	5-10-5	9	6	65
1868	1883	509999	3-10-3	47	6	258
1868	1884	510134	3-10-4	56	27	66
1869	1888	509939	5-10-5	64	29	67
1869	1884	510000	3-10-3	24	1	260
1869	1885	510135	3-10-4	70	43	68
1870	1889	505371	5-10-5	63	46	69
1870	1885	510001	3-10-3	39	12	262
1870	1886	510136	3-10-4	52	23	70
1871	1886	510002	3-10-3	10	0	264
1871	1887	510137	3-10-4	28	0	71
1872	1887	510003	3-10-3	21	0	266
1872	1888	510138	3-10-4	25	7	72
1873	1888	510004	3-10-3	21	38	269
1873	1889	510139	3-10-4	18	0	73
1874	1889	510005	3-10-3	8	0	271
1918	1933	510006	3-10-3	0	0	278
1918	1934	510140	3-10-4	81	67	74
1919	1934	510007	3-10-3	69	66	280
2270	2285	510008	3-10-3	23	0	285
2378	2397	509940	3-10-4	66	7	75
2378	2393	510009	3-10-3	23	0	290
2378	2394	510141	3-10-4	10	11	76
2379	2394	510010	3-10-3	39	6	292
2379	2395	510142	3-10-4	46	24	77
2380	2395	510011	3-10-3	33	23	294
2380	2396	510143	3-10-4	59	36	78
2381	2396	510012	3-10-3	38	22	296
2381	2397	510144	3-10-4	54	20	79
2382	2397	510013	3-10-3	42	0	298
2820	2835	510014	3-10-3	51	9	302
2820	2836	510145	3-10-4	68	19	80
2821	2836	510015	3-10-3	35	2	303
2821	2837	510146	3-10-4	65	15	81
2822	2837	510016	3-10-3	9	0	304
2822	2838	510147	3-10-4	30	0	85
2823	2838	510017	3-10-3	18	0	305
2824	2839	510018	3-10-3	24	5	306

Example 5: Dose-dependent inhibition of viral HBV RNA in HepG2.2.15 cells by MOE gapmers

Certain gapmers from the study described in Examples 3 and 4 were tested at various doses in human HepG2.2.15 cells. Cells were plated at a density of 25,000 cells per well and transfected using electroporation with 2.5 μM, 5.0 μM, 10.0 μM, and 20.0 μM concentrations of antisense oligonucleotide, as specified in Table 5. After a treatment period of approximately 16 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe set RTS3370 was used to measure mRNA levels. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented in Table 5. As illustrated in Table 5, HBV mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 5

Dose-dependent antisense inhibition of HBV RNA in HepG2.2.15 cells using RTS3370

ISIS No	2.5 μM	5.0 μM	10.0 μM	20.0 μM	IC_{50} (μM)
146786	33	50	54	81	5.7
505317	35	40	63	67	6.6
505323	16	33	48	63	11.1
505326	27	44	64	67	6.9
509929	21	44	60	62	8.4
509931	51	63	75	75	<2.5
509957	37	53	57	70	5.4
509974	25	35	54	63	9.5
509975	36	55	62	81	4.7
509981	7	23	35	52	18.8
510039	27	46	60	69	6.9
510040	10	28	43	59	13.4
510041	29	41	53	66	8.3
510058	9	34	42	63	11.9

Example 6: Dose-dependent inhibition of viral HBV RNA in HepG2.2.15 cells by MOE gapmers

Additional gapmers from the study described in Examples 3 and 4 were further tested at various doses in human HepG2.2.15 cells. Cells were plated at a density of 28,000 cells per well and transfected using LipofectAMINE 2000® reagent with 15.625 nM, 31.25 nM, 62.5 nM, 125.0 nM, and 250.0 nM

concentrations of antisense oligonucleotide, as specified in Table 6. After a treatment period of approximately 16 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe set RTS3370 was used to measure mRNA levels. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented in Table 6. As illustrated in Table 6, HBV mRNA levels were significantly reduced in a dose-dependent manner in some antisense oligonucleotide treated cells.

Table 6

Dose-dependent antisense inhibition of HBV RNA in HepG2.2.15 cells using RTS3370

ISIS No	15.625 nM	31.25 nM	62.5 nM	125.0 nM	250.0 nM	IC_{50} (nM)
146779	14	25	44	70	78	73.1
146786	10	35	64	85	93	49.4
146833	12	16	32	62	72	99.8
505317	19	31	44	69	83	65.2
505319	5	11	24	39	69	152.8
505323	2	11	26	68	90	85.4
505326	1	15	45	72	89	73.7
505327	0	4	12	56	74	128.5
505329	3	16	33	51	64	130.4
505339	26	32	59	82	92	46.0
505342	10	4	34	69	74	95.7
505347	20	26	41	70	92	63.0
505356	0	0	0	38	69	182.0
505358	8	28	47	71	84	67.9
505382	5	0	3	26	19	>250.0
509926	0	6	18	42	67	159.3
509927	3	17	33	55	76	103.2
509929	7	19	36	60	69	102.9
509931	18	28	52	76	87	57.4
509934	14	14	40	61	76	89.3
509957	20	28	51	71	79	63.1
509958	12	17	37	56	76	96.4
509959	12	11	18	59	70	121.7
509960	9	19	30	57	74	103.4
509972	15	6	17	27	45	>250.0
509974	25	35	57	83	92	45.3
509975	33	44	45	61	80	53.1

509981	0	15	11	35	60	224.4
510007	0	0	15	31	45	>250.0
510038	12	19	48	73	84	68.9
510039	17	25	44	69	72	77.3
510040	17	20	23	59	72	108.6
510041	11	21	43	64	79	80.5
510050	3	21	16	51	70	132.4
510058	7	9	16	22	46	>250.0
510079	0	6	11	29	32	>250.0
510100	18	34	50	79	83	56.1
510106	23	25	35	69	74	78.4
510116	20	44	65	79	91	42.6
510140	7	28	30	55	58	136.5

The mRNA levels were also measured with primer probe set RTS3371. The results are presented in Table 7.

Table 7

Dose-dependent antisense inhibition of HBV RNA in HepG2.2.15 cells using RTS3371

ISIS No	15.625 nM	31.25 nM	62.5 nM	125.0 nM	250.0 nM	IC_{50} (nM)
146779	16	7	38	69	68	96.9
146786	28	39	65	86	93	35
146833	26	22	52	61	65	82.3
505317	18	33	40	77	84	61.4
505319	0	0	0	15	55	>250.0
505323	0	0	33	66	87	100.5
505326	0	21	7	57	85	114.6
505327	0	0	40	50	63	132.3
505329	11	22	35	66	77	90.7
505342	15	0	1	40	59	190.1
505347	3	35	44	65	90	68.4
505356	0	0	3	42	76	153.2
505358	20	11	39	71	78	79.7
505382	0	0	0	0	0	>250.0
509926	0	4	14	55	72	130.6
509927	11	25	31	61	78	88.4
509929	11	26	41	70	77	75.8
509931	25	39	55	79	85	46.6
509934	0	25	32	54	65	119.9
509957	25	44	48	74	80	50.6
509958	24	18	20	57	72	114.5

509959	2	9	31	52	65	132.3
509960	16	28	22	57	75	101.8
509972	3	5	1	39	60	236.3
509974	38	46	65	83	94	31.2
509975	30	7	24	49	67	148.2
509981	22	22	23	46	58	194.7
510007	3	0	15	33	39	>250.0
510038	16	22	50	76	84	62.9
510039	23	36	32	70	68	79.7
510040	18	15	41	59	67	101.9
510041	0	27	38	62	81	84.5
510050	1	16	17	52	63	149
510058	20	19	40	44	51	214.1
510079	0	2	5	41	49	>250.0
510100	35	52	61	86	90	30.7
510106	27	23	5	75	81	87.9
510116	11	44	70	72	94	46.5
510140	0	18	26	45	41	>250.0

Example 7: Tolerability of MOE gapmers targeting HBV in BALB/c mice

BALB/c mice (Charles River, MA) are a multipurpose model of mice, frequently utilized for safety and efficacy testing. The mice were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for changes in the levels of various metabolic markers.

Study 1

Groups of four BALB/c mice each were injected subcutaneously twice a week for 3 weeks with 50 mg/kg of ISIS 146779, ISIS 146786, ISIS 505317, ISIS 505319, ISIS 505330, ISIS 505332, ISIS 505339, ISIS 505346, ISIS 505347, ISIS 505358, ISIS 509929, ISIS 509931, ISIS 509932, ISIS 509934, ISIS 509957, ISIS 510100, ISIS 510106, ISIS 510116, and ISIS 510140. A group of four BALB/c mice were injected subcutaneously twice a week for 3 weeks with 50 mg/kg of ISIS 141923 (CCTTCCCTGAAGGTTCCTCC (SEQ ID NO: 320)), a 5-10-5 MOE gapmer with no known homology to any human or mouse gene sequence. Another group of 4 BALB/c mice was injected subcutaneously twice a week for 3 weeks with PBS. This group of mice served as the control group. Three days after the last dose at each time point, body weights were taken, mice were euthanized and organs and plasma were harvested for further analysis.

Body and organ weights

The body weights of the mice were measured pre-dose and at the end of each treatment period. The body weights are presented in Table 8, and are expressed as percent change from the weight taken before the

start of treatment. Liver, spleen and kidney weights were measured at the end of the study, and are presented in Table 9 as a percentage difference from the respective organ weights of the PBS control. The results indicate that most of the ISIS oligonucleotides did not cause any adverse effects on body or organ weights.

Table 8

Change in body weights of BALB/c mice after antisense oligonucleotide treatment (%)

	Body weight
PBS	9
ISIS 141923	9
ISIS 146779	11
ISIS 146786	9
ISIS 505317	10
ISIS 505319	14
ISIS 505330	11
ISIS 505332	10
ISIS 505339	14
ISIS 505346	12
ISIS 505347	16
ISIS 505358	12
ISIS 509929	8
ISIS 509931	9
ISIS 509932	21
ISIS 509934	14
ISIS 509957	10
ISIS 510100	10
ISIS 510106	15
ISIS 510116	16
ISIS 510140	19

Table 9

Change in organ weights of BALB/c mice after antisense oligonucleotide treatment (%)

	Liver	Kidney	Spleen
PBS	-	-	-
ISIS 141923	3	-3	-9
ISIS 146779	10	1	13
ISIS 146786	19	-3	4
ISIS 505317	-4	-7	9
ISIS 505319	1	-16	23
ISIS 505330	12	-4	9

ISIS 505332	7	-2	14
ISIS 505339	5	-6	7
ISIS 505346	7	-6	0
ISIS 505347	12	-7	5
ISIS 505358	8	0	3
ISIS 509929	17	14	200
ISIS 509931	-4	-9	3
ISIS 509932	18	-9	79
ISIS 509934	6	-6	2
ISIS 509957	0	-2	15
ISIS 510100	2	1	8
ISIS 510106	5	-2	58
ISIS 510116	12	-8	7
ISIS 510140	20	-8	49

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Table 10 expressed in IU/L. Plasma levels of cholesterol and triglycerides were also measured using the same clinical chemistry analyzer and the results are also presented in Table 10.

Table 10

Effect of antisense oligonucleotide treatment on metabolic markers in the liver of BALB/c mice

	ALT (IU/L)	AST (IU/L)	Cholesterol (mg/dL)	Triglycerides (mg/dL)
PBS	37	58	114	238
ISIS 141923	36	57	114	234
ISIS 146779	43	56	121	221
ISIS 146786	53	76	118	327
ISIS 505317	68	103	117	206
ISIS 505319	136	152	144	168
ISIS 505330	281	194	119	188
ISIS 505332	67	70	123	226
ISIS 505339	113	111	135	249
ISIS 505346	56	63	128	234
ISIS 505347	79	83	122	347
ISIS 505358	78	175	112	214

ISIS 509929	111	166	61	175
ISIS 509931	635	508	110	179
ISIS 509932	92	113	118	131
ISIS 509934	38	89	97	176
ISIS 509957	159	229	85	173
ISIS 510100	90	87	86	222
ISIS 510106	61	88	79	239
ISIS 510116	70	95	124	214
ISIS 510140	1247	996	161	167

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 11, expressed in mg/dL.

Table 11

Effect of antisense oligonucleotide treatment on kidney markers of BALB/c mice

	BUN (mg/dL)
PBS	29
ISIS 141923	29
ISIS 146779	28
ISIS 146786	30
ISIS 505317	30
ISIS 505319	30
ISIS 505330	29
ISIS 505332	28
ISIS 505339	29
ISIS 505346	27
ISIS 505347	26
ISIS 505358	26
ISIS 509929	25
ISIS 509931	23
ISIS 509932	28
ISIS 509934	25
ISIS 509957	24
ISIS 510100	27
ISIS 510106	27
ISIS 510116	25
ISIS 510140	22

Study 2

Groups of four BALB/c mice each were injected subcutaneously twice a week for 3 weeks with 50 mg/kg of ISIS 505329, ISIS 509926, ISIS 509927, ISIS 509958, ISIS 509959, ISIS 509960, ISIS 509974, ISIS 509975, ISIS 510038, ISIS 510039, ISIS 510040, ISIS 510041, and ISIS 510050. A group of 4 BALB/c mice was injected subcutaneously twice a week for 3 weeks with PBS. This group of mice served as the control group. Three days after the last dose at each time point, body weights were taken, mice were euthanized and organs and plasma were harvested for further analysis.

Organ weights

Liver, spleen and kidney weights were measured at the end of the study, and are also presented in Table 12 as a percentage change over the respective organ weights of the PBS control.

Table 12

Change in organ weights of BALB/c mice after antisense oligonucleotide treatment (%)

ISIS No	Liver	Kidney	Spleen
505329	12	2	12
509926	23	3	30
509927	8	-4	27
509958	1	-4	9
509959	7	0	26
509960	16	6	30
509974	5	8	7
509975	1	-1	7
510038	6	4	23
510039	0	15	9
510040	3	1	2
510041	6	6	10
510050	5	5	18

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Table 13 expressed in IU/L.

Table 13

Effect of antisense oligonucleotide treatment on transaminases (IU/L) in the liver of BALB/c mice

	ALT	AST
PBS	37	78
ISIS 505329	48	65
ISIS 509926	77	120
ISIS 509927	71	92
ISIS 509958	106	105
ISIS 509959	119	122
ISIS 509960	40	66
ISIS 509974	38	43
ISIS 509975	33	45
ISIS 510038	69	66
ISIS 510039	32	61
ISIS 510040	83	113
ISIS 510041	32	45
ISIS 510050	26	47

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in Table 14, expressed in mg/dL.

Table 14

Effect of antisense oligonucleotide treatment on kidney markers of BALB/c mice

	BUN
PBS	21
ISIS 505329	22
ISIS 509926	20
ISIS 509927	20
ISIS 509958	22
ISIS 509959	21
ISIS 509960	20
ISIS 509974	19
ISIS 509975	19
ISIS 510038	19
ISIS 510039	19
ISIS 510040	22
ISIS 510041	18

ISIS 510050	22

Example 8: Dose response confirmation of MOE gapmers targeting HBV in HepG2.2.15 cells

Gapmers were chosen based on sequence conservation, activity and tolerability, as measured in the study described in Examples 7 and 8, and tested at various doses in HepG2.2.15 cells. Cells were plated at a density of 28,000 cells per well and transfected using LipofectAMINE 2000 reagent with 15.625 nM, 31.25 nM, 62.5 nM, 125.0 nM and 250.0 nM concentrations of antisense oligonucleotide. Two days post-transfection, the media was replaced with fresh media. Samples were collected 4 days post-transfection. DNA, RNA, HBsAg and HBeAg levels were measured in the supernatant.

HBV mRNA levels were measured by quantitative real-time PCR. HBV primer probe set RTS3370 was used to measure mRNA levels. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells. As illustrated in Table 15, HBV mRNA levels were reduced in a dose-dependent manner in most of the antisense oligonucleotide treated cells.

HBV antigens in the supernatants were detected with the ELISA technique. HBs antigen (HBsAg) levels were detected by ELISA from Abazyme LLC, MA. As presented in Table 16, treatment with ISIS oligonucleotides 146779, 146786, 505329, 505330, 505339, 505347, 505358, 509927, 509934, 509958, 509959, 509960, 509974, 5100038, 510039, 510040, 510041, 510100, 510106, and 510116 caused significant reduction in HBsAg levels. HBe antigen (HBeAg) levels were detected by ELISA from International Immuno-diagnostics, CA. As presented in Table 17, treatment with ISIS oligonucleotides 146779, 146786, 505329, 505330, 505339, 505347, 505358, 509927, 509934, 509958, 509959, 509960, 509974, 5100038, 510039, 510040, 510041, 510100, 510106, and 510116 caused significant reduction in HBeAg levels. HBV DNA levels were measured using primer probe set RTS3370. As presented in Table 18, treatment with ISIS oligonucleotides 146779, 146786, 505329, 505330, 505339, 505347, 505358, 509927, 509934, 509958, 509959, 509960, 509974, 5100038, 510039, 510040, 510041, 510100, 510106, and 510116 caused significant reduction in HBV DNA levels. The total protein in the supernatants was measured by a DC protein assay (BioRad), as presented in Table 19.

Table 15

Dose-dependent antisense inhibition of HBV RNA in **HepG2.2.15**cells

ISIS No	15.625nM	31.25nM	62.5nM	125nM	250nM
146779	10	25	42	64	95
146786	23	59	78	84	90
505329	45	49	57	69	83
505330	31	61	65	80	93

505339	31	56	78	89	97
505347	30	50	72	87	96
505358	28	52	75	86	95
509927	41	61	67	61	76
509934	38	61	64	82	58
509958	50	67	72	79	89
509959	50	63	73	80	86
509960	63	61	72	82	74
509974	29	44	75	91	96
510038	29	40	85	89	93
510039	32	34	63	84	84
510040	18	0	51	71	77
510041	34	53	67	76	71
510100	29	64	70	89	93
510106	28	65	64	81	85
510116	13	34	78	89	95

Table 16

Dose-dependent reduction of S antigen in HepG2.2.15 cell supernatant

ISIS No	15.625nM	31.25nM	62.5nM	125nM
146779	40	58	80	92
146786	47	75	92	98
505329	37	58	71	89
505330	45	66	84	95
505339	62	79	93	96
505347	68	71	89	97
505358	69	83	92	96
509927	54	74	88	94
509934	40	59	78	89
509958	57	77	91	93
509959	54	72	84	100
509960	44	72	91	91
509974	58	77	92	95
510038	58	78	94	98
510039	53	74	89	95
510040	39	70	80	90
510041	47	65	82	92
510100	74	83	95	96
510106	54	75	86	92
510116	61	74	91	94

Table 17

Dose-dependent reduction of E antigen in HepG2.2.15 cell supernatant

ISIS No	15.625nM	31.25nM	62.5nM	125nM
146779	14	45	66	76
146786	26	58	75	80
505329	19	26	60	73
505330	28	70	69	80
505339	31	57	77	82
505347	24	33	64	77
505358	26	45	72	81
509927	34	54	72	79
509934	21	42	59	73
509958	29	45	72	77
509959	60	64	77	80
509960	19	36	67	77
509974	16	48	72	80
510038	20	35	79	80
510039	14	41	64	78
510040	0	8	37	69
510041	9	34	63	76
510100	26	52	73	81
510106	7	42	62	76
510116	27	56	76	81

Table 18

Dose-dependent antisense inhibition of HBV DNA in HepG2.2.15 cells

ISIS No	15.625nM	31.25nM	62.5nM	125nM
146779	71	71	84	85
146786	67	81	82	75
505329	53	65	72	67
505330	72	76	86	90
505339	83	85	89	88
505347	76	78	81	87
505358	79	82	90	87
509927	51	75	78	69
509934	61	60	64	75
509958	57	73	69	71
509959	59	54	73	73
509960	48	66	63	54
509974	76	90	84	85

510038	69	76	90	87
510039	70	79	81	86
510040	40	67	68	68
510041	53	71	62	68
510100	76	81	87	87
510106	46	74	73	76
510116	79	84	89	86

Table 19

Total protein levels in HepG2.2.15 cell supernatant

	15.625nM	31.25nM	62.5nM	125nM
PBS	5601	5601	5601	5601
146779	6491	6631	6027	5067
146786	5408	5328	4839	3518
505329	5719	5285	5384	4994
505330	7514	7262	6627	5179
505339	6572	6343	5349	4550
505347	7315	6602	6378	5908
505358	6357	6871	5798	5720
509927	5581	5487	5145	3601
509934	5476	5610	5394	4127
509958	5193	5492	5071	3957
509959	5051	5312	5144	3893
509960	4726	5160	5071	3305
509974	6913	7624	5798	5389
510038	5707	6381	5772	6733
510039	5981	7629	4802	6156
510040	4302	5209	5049	4188
510041	5565	5607	5205	3757
510100	8466	8378	7985	6402
510106	5703	5940	5231	4005
510116	5880	5380	4797	4757

Example 9: *In vivo* inhibition of HBV mRNA by MOE gapmers in HBV-transgenic mice

ISIS 146786, a 5-10-5 MOE gapmer, and ISIS 510100, a 3-10-4 MOE gapmer, both demonstrating significant inhibition of HBV mRNA, were tested in transgenic mice containing the HBV gene (Chisari 1.3.32 line) (Guidotti, L. G. et al., *J. Virol.* **1995**, *69*, 6158-6169) and the efficacy of the gapmers was evaluated.

Treatment

Two groups of ten-eleven HBV-transgenic male and female mice each were administered subcutaneously twice a week for four weeks with 25 mg/kg of ISIS 146786 or ISIS 510100. Another group of 14 male and female HBV-transgenic mice was administered Entecavir, an oral antiviral drug used to treat Hepatitis B infection, at 1 mg/kg daily for two weeks. Another group of 10 male and female HBV-transgenic female mice were injected subcutaneously with PBS twice a week for four weeks. The mice injected with PBS served as a control group. Liver HBV mRNA and DNA levels, plasma ALT, and body and organ weights were measured.

RNA analysis

RNA was extracted from liver tissue for real-time PCR analysis of HBV using primer probe sets RTS3370, RTS3371, and RTS3372. Results are presented as percent inhibition of HBV mRNA, relative to PBS control. As shown in Table 20, treatment with ISIS antisense oligonucleotides resulted in significant reduction of HBV mRNA in comparison to the PBS control, irrespective of the primer probe set used for measurement. Entecavir did not decrease HBV mRNA expression.

Table 20

Inhibition of HBV mRNA in HBV-transgenic mice liver relative to the PBS control

ISIS No	RTS3370	RTS3371	RTS3372
146786	82	75	81
510100	93	83	89

DNA analysis

DNA was extracted from liver tissue for real-time PCR analysis of HBV using primer probe sets RTS3370 and RTS3371. The levels were normalized to RIBOGREEN®. Results are presented as percent inhibition of HBV DNA, relative to PBS control. As shown in Table 21, treatment with ISIS antisense oligonucleotides resulted in significant reduction of HBV DNA in comparison to the PBS control, irrespective of the primer probe set used for measurement. Treatment with Entecavir also reduced DNA levels, as expected.

Table 21

Inhibition of HBV DNA in HBV-transgenic mice liver relative to the PBS control

ISIS No	RTS3370	RTS3371
146786	65	69
510100	67	73
Entecavir	75	96

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminase was measured using a manual clinical chemistry analyzer (Teco Diagnostics, Anaheim, CA)Plasma concentrations of ALT (alanine transaminase) were measured and the results are presented in Table 22, expressed in IU/L. The results indicate that antisense inhibition of HBV had no adverse effects on the liver function of the mice.

Table 22

Effect of antisense oligonucleotide treatment on liver ALT of transgenic mice

	IU/mL
PBS	12.7
ISIS 146786	24.1
ISIS 510100	25.8
Entecavir	23.7

The data from the study indicates that both ISIS 146786 and ISIS 510100 caused robust reductions in liver HBV RNA and DNA and treatment with these oligonucleotides were well tolerated in the transgenic mice.

Example 10: Antisense inhibition of HBV viral mRNA in HepG2.2.15 cells by MOE gapmers

Additional antisense oligonucleotides were designed targeting a HBV viral nucleic acid and were tested for their effects on HBV mRNA in vitro. Several of the antisense oligonucleotides from the studies described above were also included in the assay. Cultured HepG2.2.15 cells at a density of 28,000 cells per well were transfected using LipofectAMINE 2000® reagent with 100 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe set RTS3370 was used to measure mRNA levels. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in Table 23 were designed as 5-10-5 MOE gapmers. The gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has an MOE sugar modification. Each nucleoside in the central gap segment has a deoxy sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines.

"Viral Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the viral gene sequence. "Viral Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted viral gene sequence. Each gapmer listed in Table 23 is targeted to the viral genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. U95551.1).

Table 23

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1 (RTS3370)

Viral Start Site	Viral Stop Site	ISIS No	Sequence	% inhibition	SEQ ID NO
1	20	524410	TGGTGAAAGGTTGTGGAATT	70	321
4	23	524411	GTTTGGTGAAAGGTTGTGGA	51	322
7	26	524412	AGAGTTTGGTGAAAGGTTGT	47	323
10	29	524413	TGCAGAGTTTGGTGAAAGGT	74	324
13	32	524414	TCTTGCAGAGTTTGGTGAAA	91	325
16	35	524415	GGATCTTGCAGAGTTTGGTG	93	326
19	38	524416	CTGGGATCTTGCAGAGTTTG	85	327
22	41	524417	ACTCTGGGATCTTGCAGAGT	66	328
25	44	524418	CTCACTCTGGGATCTTGCAG	86	329
28	47	524419	CCTCTCACTCTGGGATCTTG	81	330
31	50	524420	AGGCCTCTCACTCTGGGATC	77	331
34	53	524421	TACAGGCCTCTCACTCTGGG	71	332
37	56	524422	AAATACAGGCCTCTCACTCT	68	333
40	59	524423	GGGAAATACAGGCCTCTCAC	43	334
43	62	524424	GCAGGGAAATACAGGCCTCT	76	335
46	65	524425	CCAGCAGGGAAATACAGGCC	89	336
49	68	524426	CCACCAGCAGGGAAATACAG	82	337
52	71	524427	GAGCCACCAGCAGGGAAATA	53	338
55	74	524428	CTGGAGCCACCAGCAGGGAA	76	339
56	75	524429	ACTGGAGCCACCAGCAGGGA	55	340
57	76	524430	AACTGGAGCCACCAGCAGGG	45	341
58	77	146779	GAACTGGAGCCACCAGCAGG	57	83
59	78	524431	TGAACTGGAGCCACCAGCAG	85	342
60	79	524432	CTGAACTGGAGCCACCAGCA	90	343
61	80	505314	CCTGAACTGGAGCCACCAGC	93	85
62	81	524433	TCCTGAACTGGAGCCACCAG	79	344
63	82	524434	CTCCTGAACTGGAGCCACCA	82	345
65	84	524435	TGCTCCTGAACTGGAGCCAC	78	346
68	87	524436	TACTGCTCCTGAACTGGAGC	58	347
71	90	524437	GTTTACTGCTCCTGAACTGG	40	348
74	93	524438	AGGGTTTACTGCTCCTGAAC	45	349

77	96	524439	AACAGGGTTTACTGCTCCTG	69	350
80	99	524440	CGGAACAGGGTTTACTGCTC	67	351
83	102	524441	AGTCGGAACAGGGTTTACTG	47	352
86	105	524442	AGTAGTCGGAACAGGGTTTA	59	353
89	108	524443	GGCAGTAGTCGGAACAGGGT	47	354
92	111	524444	AGAGGCAGTAGTCGGAACAG	54	355
95	114	524445	GGGAGAGGCAGTAGTCGGAA	49	356
98	117	524446	TAAGGGAGAGGCAGTAGTCG	81	357
101	120	524447	CGATAAGGGAGAGGCAGTAG	86	358
104	123	524448	TGACGATAAGGGAGAGGCAG	79	359
107	126	524449	GATTGACGATAAGGGAGAGG	27	360
110	129	524450	GAAGATTGACGATAAGGGAG	53	361
113	132	524451	CGAGAAGATTGACGATAAGG	67	362
116	135	524452	CCTCGAGAAGATTGACGATA	84	363
119	138	524453	AATCCTCGAGAAGATTGACG	79	364
122	141	524454	CCCAATCCTCGAGAAGATTG	65	365
125	144	524455	GTCCCCAATCCTCGAGAAGA	66	366
128	147	524456	AGGGTCCCCAATCCTCGAGA	67	367
131	150	524457	CGCAGGGTCCCCAATCCTCG	76	368
134	153	524458	CAGCGCAGGGTCCCCAATCC	59	369
137	156	524459	GTTCAGCGCAGGGTCCCCAA	80	370
140	159	524460	CATGTTCAGCGCAGGGTCCC	90	371
143	162	524461	CTCCATGTTCAGCGCAGGGT	75	372
146	165	524462	GTTCTCCATGTTCAGCGCAG	54	373
149	168	524463	GATGTTCTCCATGTTCAGCG	27	374
152	171	524464	TGTGATGTTCTCCATGTTCA	72	375
158	177	524466	TCCTGATGTGATGTTCTCCA	91	376
161	180	524467	GAATCCTGATGTGATGTTCT	77	377
164	183	524468	TAGGAATCCTGATGTGATGT	77	378
167	186	524469	TCCTAGGAATCCTGATGTGA	94	379
170	189	524470	GGGTCCTAGGAATCCTGATG	56	380
188	207	524471	CGCCTGTAACACGAGAAGGG	65	381
191	210	524472	CCCCGCCTGTAACACGAGAA	71	382
194	213	524473	AAACCCCGCCTGTAACACGA	74	383
195	214	524474	AAAACCCCGCCTGTAACACG	72	384
196	215	505315	AAAAACCCCGCCTGTAACAC	52	87
197	216	524475	GAAAAACCCCGCCTGTAACA	38	385
198	217	524476	AGAAAAACCCCGCCTGTAAC	18	386
200	219	524477	CAAGAAAAACCCCGCCTGTA	86	387
203	222	524478	CAACAAGAAAAACCCCGCCT	84	388
204	223	524479	TCAACAAGAAAAACCCCGCC	80	389
205	224	505317	GTCAACAAGAAAAACCCCGC	84	89
206	225	524480	TGTCAACAAGAAAAACCCCG	79	390

207	226	524481	TTGTCAACAAGAAAAACCCC	76	391
209	228	524482	TCTTGTCAACAAGAAAAACC	86	392
212	231	524483	GATTCTTGTCAACAAGAAAA	57	393
215	234	524484	GAGGATTCTTGTCAACAAGA	51	394
218	237	524485	TGTGAGGATTCTTGTCAACA	83	395
221	240	524486	TATTGTGAGGATTCTTGTCA	61	396
224	243	524487	CGGTATTGTGAGGATTCTTG	74	397
227	246	524488	CTGCGGTATTGTGAGGATTC	49	398
230	249	524489	ACTCTGCGGTATTGTGAGGA	67	399
233	252	524490	TAGACTCTGCGGTATTGTGA	88	400
236	255	524491	GTCTAGACTCTGCGGTATTG	84	401
239	258	524492	CGAGTCTAGACTCTGCGGTA	82	402
242	261	524493	CCACGAGTCTAGACTCTGCG	94	403
243	262	524494	ACCACGAGTCTAGACTCTGC	87	404
244	263	146821	CACCACGAGTCTAGACTCTG	87	92
245	264	524495	CCACCACGAGTCTAGACTCT	80	405
246	265	524496	TCCACCACGAGTCTAGACTC	65	406
247	266	505318	GTCCACCACGAGTCTAGACT	65	96
248	267	524497	AGTCCACCACGAGTCTAGAC	46	407
249	268	524498	AAGTCCACCACGAGTCTAGA	54	408
250	269	509921	GAAGTCCACCACGAGTCTAG	35	97
251	270	509922	AGAAGTCCACCACGAGTCTA	51	99
252	271	509923	GAGAAGTCCACCACGAGTCT	49	101
253	272	505319	AGAGAAGTCCACCACGAGTC	60	103
254	273	509924	GAGAGAAGTCCACCACGAGT	46	105
255	274	509925	TGAGAGAAGTCCACCACGAG	79	108
256	275	505320	TTGAGAGAAGTCCACCACGA	84	111
257	276	524499	ATTGAGAGAAGTCCACCACG	83	409
260	279	524500	AAAATTGAGAGAAGTCCACC	71	410
263	282	524501	TAGAAAATTGAGAGAAGTCC	67	411
266	285	524502	CCCTAGAAAATTGAGAGAAG	88	412
269	288	524503	TCCCCCTAGAAAATTGAGAG	82	413
272	291	524504	AGTTCCCCCTAGAAAATTGA	66	414
275	294	524505	GGTAGTTCCCCCTAGAAAAT	0	415
278	297	524506	CACGGTAGTTCCCCCTAGAA	65	416
281	300	524507	ACACACGGTAGTTCCCCCTA	87	417
284	303	524508	AAGACACACGGTAGTTCCCC	76	418
287	306	524509	GCCAAGACACACGGTAGTTC	61	419
290	309	524510	TTGGCCAAGACACACGGTAG	87	420
291	310	524511	TTTGGCCAAGACACACGGTA	87	421
292	311	524512	TTTTGGCCAAGACACACGGT	93	422
293	312	505323	ATTTTGGCCAAGACACACGG	83	123
294	313	524513	AATTTTGGCCAAGACACACG	79	423

295	314	524514	GAATTTTGGCCAAGACACAC	74	424
298	317	524515	TGCGAATTTTGGCCAAGACA	78	425
300	319	524516	ACTGCGAATTTTGGCCAAGA	71	426
301	320	524517	GACTGCGAATTTTGGCCAAG	71	427
302	321	505325	GGACTGCGAATTTTGGCCAA	50	125
303	322	524518	GGGACTGCGAATTTTGGCCA	55	428
321	340	524519	GTGAGTGATTGGAGGTTGGG	68	429
324	343	524520	TTGGTGAGTGATTGGAGGTT	84	430
327	346	524521	AGGTTGGTGAGTGATTGGAG	64	431
330	349	524522	AGGAGGTTGGTGAGTGATTG	58	432
333	352	524523	GACAGGAGGTTGGTGAGTGA	62	433
336	355	524524	GAGGACAGGAGGTTGGTGAG	56	434
339	358	524525	TTGGAGGACAGGAGGTTGGT	81	435
342	361	524526	AAGTTGGAGGACAGGAGGTT	77	436
345	364	524527	GACAAGTTGGAGGACAGGAG	69	437
348	367	524528	CAGGACAAGTTGGAGGACAG	82	438
351	370	524529	AACCAGGACAAGTTGGAGGA	67	439
354	373	524530	GATAACCAGGACAAGTTGGA	53	440
357	376	524531	AGCGATAACCAGGACAAGTT	55	441
358	377	524532	CAGCGATAACCAGGACAAGT	84	442
359	378	524533	CCAGCGATAACCAGGACAAG	86	443
360	379	505326	TCCAGCGATAACCAGGACAA	79	126
361	380	524534	ATCCAGCGATAACCAGGACA	85	444
362	381	524535	CATCCAGCGATAACCAGGAC	90	445
364	383	524536	CACATCCAGCGATAACCAGG	82	446
365	384	524537	ACACATCCAGCGATAACCAG	72	447
366	385	505327	GACACATCCAGCGATAACCA	61	127
367	386	524538	AGACACATCCAGCGATAACC	79	448
368	387	524539	CAGACACATCCAGCGATAAC	73	449
370	389	524540	CGCAGACACATCCAGCGATA	94	450
373	392	524541	CGCCGCAGACACATCCAGCG	84	451
390	409	524542	AGAGGAAGATGATAAAACGC	45	452
393	412	524543	TGAAGAGGAAGATGATAAAA	62	453
396	415	524544	GGATGAAGAGGAAGATGATA	58	454
399	418	524545	GCAGGATGAAGAGGAAGATG	48	455
402	421	524546	GCAGCAGGATGAAGAGGAAG	60	456
405	424	524547	ATAGCAGCAGGATGAAGAGG	84	457
408	427	524548	GGCATAGCAGCAGGATGAAG	56	458
409	428	524549	AGGCATAGCAGCAGGATGAA	78	459
410	429	524550	GAGGCATAGCAGCAGGATGA	67	460
411	430	505329	TGAGGCATAGCAGCAGGATG	85	136
412	431	509926	ATGAGGCATAGCAGCAGGAT	84	139
413	432	509927	GATGAGGCATAGCAGCAGGA	68	142

414	433	505330	AGATGAGGCATAGCAGCAGG	82	20
415	434	509928	AAGATGAGGCATAGCAGCAG	83	22
416	435	509929	GAAGATGAGGCATAGCAGCA	80	24
417	436	509930	AGAAGATGAGGCATAGCAGC	78	26
418	437	146783	AAGAAGATGAGGCATAGCAG	80	28
419	438	524551	CAAGAAGATGAGGCATAGCA	55	461
422	441	524552	CAACAAGAAGATGAGGCATA	90	462
425	444	524553	AACCAACAAGAAGATGAGGC	82	463
428	447	524554	AAGAACCAACAAGAAGATGA	79	464
431	450	524555	CAGAAGAACCAACAAGAAGA	72	465
434	453	524556	GTCCAGAAGAACCAACAAGA	87	466
437	456	524557	ATAGTCCAGAAGAACCAACA	72	467
440	459	524558	TTGATAGTCCAGAAGAACCA	76	468
443	462	524559	ACCTTGATAGTCCAGAAGAA	78	469
446	465	524560	CATACCTTGATAGTCCAGAA	77	470
449	468	524561	CAACATACCTTGATAGTCCA	69	471
452	471	524562	GGGCAACATACCTTGATAGT	39	472
455	474	524563	AACGGGCAACATACCTTGAT	72	473
456	475	524564	AAACGGGCAACATACCTTGA	86	474
457	476	505332	CAAACGGGCAACATACCTTG	85	166
458	477	524565	ACAAACGGGCAACATACCTT	80	475
459	478	524566	GACAAACGGGCAACATACCT	42	476
461	480	524567	AGGACAAACGGGCAACATAC	47	477
464	483	524568	TAGAGGACAAACGGGCAACA	81	478
467	486	524569	AATTAGAGGACAAACGGGCA	72	479
470	489	524570	TGGAATTAGAGGACAAACGG	84	480
471	490	524571	CTGGAATTAGAGGACAAACG	86	481
472	491	505335	CCTGGAATTAGAGGACAAAC	89	174
473	492	524572	TCCTGGAATTAGAGGACAAA	92	482
474	493	524573	ATCCTGGAATTAGAGGACAA	86	483
476	495	524574	GGATCCTGGAATTAGAGGAC	76	484
479	498	524575	TGAGGATCCTGGAATTAGAG	77	485
482	501	524576	GGTTGAGGATCCTGGAATTA	62	486
485	504	524577	GGTGGTTGAGGATCCTGGAA	73	487
488	507	524578	GCTGGTGGTTGAGGATCCTG	84	488
491	510	524579	CGTGCTGGTGGTTGAGGATC	79	489
494	513	524580	TCCCGTGCTGGTGGTTGAGG	83	490
497	516	524581	TGGTCCCGTGCTGGTGGTTG	66	491
500	519	524582	GCATGGTCCCGTGCTGGTGG	77	492
503	522	524583	TCGGCATGGTCCCGTGCTGG	0	493
506	525	524584	GGTTCGGCATGGTCCCGTGC	56	494
509	528	524585	GCAGGTTCGGCATGGTCCCG	61	495
512	531	524586	CATGCAGGTTCGGCATGGTC	87	496

515	534	524587	AGTCATGCAGGTTCGGCATG	77	497
518	537	524588	AGTAGTCATGCAGGTTCGGC	64	498
521	540	524589	AGCAGTAGTCATGCAGGTTC	61	499
524	543	524590	TTGAGCAGTAGTCATGCAGG	86	500
527	546	524591	TCCTTGAGCAGTAGTCATGC	80	501
530	549	524592	GGTTCCTTGAGCAGTAGTCA	50	502
533	552	524593	AGAGGTTCCTTGAGCAGTAG	61	503
536	555	524594	CATAGAGGTTCCTTGAGCAG	89	504
539	558	524595	ATACATAGAGGTTCCTTGAG	87	505
542	561	524596	GGGATACATAGAGGTTCCTT	0	506
545	564	524597	GGAGGGATACATAGAGGTTC	38	507
548	567	524598	ACAGGAGGGATACATAGAGG	73	508
551	570	524599	GCAACAGGAGGGATACATAG	67	509
554	573	524600	ACAGCAACAGGAGGGATACA	72	510
557	576	524601	GGTACAGCAACAGGAGGGAT	59	511
560	579	524602	TTTGGTACAGCAACAGGAGG	81	512
563	582	524603	AGGTTTGGTACAGCAACAGG	74	513
566	585	524604	CGAAGGTTTGGTACAGCAAC	85	514
569	588	524605	GTCCGAAGGTTTGGTACAGC	76	515
572	591	524606	TCCGTCCGAAGGTTTGGTAC	80	516
575	594	524607	ATTTCCGTCCGAAGGTTTGG	88	517
578	597	524608	GCAATTTCCGTCCGAAGGTT	50	518
581	600	524609	GGTGCAATTTCCGTCCGAAG	55	519
584	603	524610	ACAGGTGCAATTTCCGTCCG	81	520
587	606	524611	AATACAGGTGCAATTTCCGT	88	521
590	609	524612	GGGAATACAGGTGCAATTTC	32	522
593	612	524613	GATGGGAATACAGGTGCAAT	49	523
608	627	524614	AGCCCAGGATGATGGGATGG	89	524
611	630	524615	GAAAGCCCAGGATGATGGGA	71	525
614	633	524616	TCCGAAAGCCCAGGATGATG	86	526
617	636	524617	TTTTCCGAAAGCCCAGGATG	97	527
620	639	524618	GAATTTTCCGAAAGCCCAGG	80	528
623	642	524619	TAGGAATTTTCCGAAAGCCC	95	529
626	645	524620	CCATAGGAATTTTCCGAAAG	88	530
629	648	524621	CTCCCATAGGAATTTTCCGA	83	531
632	651	524622	CCACTCCCATAGGAATTTTC	68	532
635	654	524623	GGCCCACTCCCATAGGAATT	60	533
638	657	524624	TGAGGCCCACTCCCATAGGA	57	534
641	660	524625	GGCTGAGGCCCACTCCCATA	62	535
644	663	524626	ACGGGCTGAGGCCCACTCCC	57	536
647	666	524627	GAAACGGGCTGAGGCCCACT	62	537
650	669	524628	GGAGAAACGGGCTGAGGCCC	31	538
653	672	524629	CCAGGAGAAACGGGCTGAGG	77	539

656	675	524630	GAGCCAGGAGAAACGGGCTG	48	540
659	678	524631	ACTGAGCCAGGAGAAACGGG	43	541
662	681	524632	TAAACTGAGCCAGGAGAAAC	67	542
665	684	524633	TAGTAAACTGAGCCAGGAGA	86	543
668	687	524634	CACTAGTAAACTGAGCCAGG	96	544
669	688	524635	GCACTAGTAAACTGAGCCAG	83	545
671	690	524636	TGGCACTAGTAAACTGAGCC	84	546
672	691	524637	ATGGCACTAGTAAACTGAGC	82	547
674	693	524638	AAATGGCACTAGTAAACTGA	74	548
677	696	524639	AACAAATGGCACTAGTAAAC	63	549
678	697	524640	GAACAAATGGCACTAGTAAA	67	550
679	698	505338	TGAACAAATGGCACTAGTAA	84	186
680	699	524641	CTGAACAAATGGCACTAGTA	95	551
681	700	524642	ACTGAACAAATGGCACTAGT	77	552
682	701	505339	CACTGAACAAATGGCACTAG	95	187
683	702	524643	CCACTGAACAAATGGCACTA	89	553
684	703	524644	ACCACTGAACAAATGGCACT	90	554
686	705	524646	GAACCACTGAACAAATGGCA	82	555
687	706	509931	CGAACCACTGAACAAATGGC	90	39
689	708	524647	TACGAACCACTGAACAAATG	79	556
690	709	146824	CTACGAACCACTGAACAAAT	72	557
692	711	524648	CCCTACGAACCACTGAACAA	73	558
693	712	524649	GCCCTACGAACCACTGAACA	83	559
695	714	524650	AAGCCCTACGAACCACTGAA	82	560
696	715	524651	AAAGCCCTACGAACCACTGA	81	561
697	716	505342	GAAAGCCCTACGAACCACTG	66	198
698	717	524652	GGAAAGCCCTACGAACCACT	59	562
699	718	524653	GGGAAAGCCCTACGAACCAC	46	563
718	737	524654	ACTGAAAGCCAAACAGTGGG	64	564
721	740	524655	ATAACTGAAAGCCAAACAGT	0	565
724	743	524656	CATATAACTGAAAGCCAAAC	70	566
727	746	524657	ATCCATATAACTGAAAGCCA	91	567
730	749	524658	ATCATCCATATAACTGAAAG	69	568
733	752	524659	CACATCATCCATATAACTGA	70	569
736	755	524660	TACCACATCATCCATATAAC	57	570
739	758	524661	CAATACCACATCATCCATAT	70	571
742	761	524662	CCCCAATACCACATCATCCA	85	572
745	764	524663	GGCCCCCAATACCACATCAT	70	573
748	767	524664	CTTGGCCCCCAATACCACAT	82	574
751	770	524665	AGACTTGGCCCCCAATACCA	77	575
754	773	524666	TACAGACTTGGCCCCCAATA	77	576
757	776	524667	CTGTACAGACTTGGCCCCCA	90	577
760	779	524668	ATGCTGTACAGACTTGGCCC	79	578

763	782	524669	AAGATGCTGTACAGACTTGG	79	579
766	785	524670	CTCAAGATGCTGTACAGACT	84	580
769	788	524671	GGACTCAAGATGCTGTACAG	24	581
772	791	524672	AAGGGACTCAAGATGCTGTA	57	582
775	794	524673	AAAAAGGGACTCAAGATGCT	66	583
778	797	524674	GGTAAAAAGGGACTCAAGAT	30	584
781	800	524675	AGCGGTAAAAAGGGACTCAA	68	585
784	803	524676	AACAGCGGTAAAAAGGGACT	67	586
787	806	524677	GGTAACAGCGGTAAAAAGGG	48	587
790	809	524678	ATTGGTAACAGCGGTAAAAA	81	588
793	812	524679	AAAATTGGTAACAGCGGTAA	89	589
796	815	524680	AAGAAAATTGGTAACAGCGG	84	590
799	818	524681	CAAAAGAAAATTGGTAACAG	41	591
802	821	524682	AGACAAAAGAAAATTGGTAA	51	592
805	824	524683	CAAAGACAAAAGAAAATTGG	66	593
808	827	524684	ACCCAAAGACAAAAGAAAAT	61	594
811	830	524685	TATACCCAAAGACAAAAGAA	79	595
814	833	524686	ATGTATACCCAAAGACAAAA	84	596
817	836	524687	TAAATGTATACCCAAAGACA	77	597
820	839	524688	GTTTAAATGTATACCCAAAG	80	598
821	840	524689	GGTTTAAATGTATACCCAAA	71	599
822	841	524690	GGGTTTAAATGTATACCCAA	85	600
823	842	505344	AGGGTTTAAATGTATACCCA	85	206
824	843	524691	TAGGGTTTAAATGTATACCC	90	601
825	844	524692	TTAGGGTTTAAATGTATACC	83	602
827	846	524693	TGTTAGGGTTTAAATGTATA	53	603
830	849	524694	TTTTGTTAGGGTTTAAATGT	67	604
845	864	524695	AACCCCATCTCTTTGTTTTG	81	605
848	867	524696	AGTAACCCCATCTCTTTGTT	71	606
851	870	524697	GAGAGTAACCCCATCTCTTT	65	607
854	873	524698	TCAGAGAGTAACCCCATCTC	96	608
857	876	524699	AATTCAGAGAGTAACCCCAT	94	609
860	879	524700	TAAAATTCAGAGAGTAACCC	71	610
863	882	524701	CCATAAAATTCAGAGAGTAA	90	611
866	885	524702	AACCCATAAAATTCAGAGAG	86	612
869	888	524703	CATAACCCATAAAATTCAGA	72	613
872	891	524704	TGACATAACCCATAAAATTC	81	614
875	894	524705	CAATGACATAACCCATAAAA	81	615
878	897	524706	TTCCAATGACATAACCCATA	95	616
881	900	524707	AACTTCCAATGACATAACCC	91	617
884	903	524708	CATAACTTCCAATGACATAA	83	618
887	906	524709	ACCCATAACTTCCAATGACA	95	619
890	909	524710	AGGACCCATAACTTCCAATG	66	620

893	912	524711	GCAAGGACCCATAACTTCCA	41	621
896	915	524712	GTGGCAAGGACCCATAACTT	53	622
899	918	524713	CTTGTGGCAAGGACCCATAA	91	623
902	921	524714	GTTCTTGTGGCAAGGACCCA	77	624
905	924	524715	TGTGTTCTTGTGGCAAGGAC	90	625
908	927	524716	TGATGTGTTCTTGTGGCAAG	90	626
911	930	524717	GTATGATGTGTTCTTGTGGC	82	627
914	933	524718	TTTGTATGATGTGTTCTTGT	95	628
930	949	524719	AAACATTCTTTGATTTTTTG	61	629
933	952	524720	CTAAAACATTCTTTGATTTT	43	630
936	955	524721	TTTCTAAAACATTCTTTGAT	90	631
939	958	524722	AGTTTTCTAAAACATTCTTT	75	632
942	961	524723	GGAAGTTTTCTAAAACATTC	52	633
945	964	524724	ATAGGAAGTTTTCTAAAACA	74	634
948	967	524725	TTAATAGGAAGTTTTCTAAA	40	635
951	970	524726	CTGTTAATAGGAAGTTTTCT	93	636
954	973	524727	GGCCTGTTAATAGGAAGTTT	87	637
957	976	524728	ATAGGCCTGTTAATAGGAAG	85	638
960	979	524729	TCAATAGGCCTGTTAATAGG	92	639
963	982	524730	CAATCAATAGGCCTGTTAAT	90	640
966	985	524731	TTCCAATCAATAGGCCTGTT	96	641
969	988	524732	ACTTTCCAATCAATAGGCCT	77	642
972	991	146826	CATACTTTCCAATCAATAGG	92	643
975	994	524733	TGACATACTTTCCAATCAAT	91	644
978	997	524734	CGTTGACATACTTTCCAATC	95	645
996	1015	524735	CCCAAAAGACCCACAATTCG	92	646
999	1018	524736	AAACCCAAAAGACCCACAAT	74	647
1002	1021	524737	GCAAAACCCAAAAGACCCAC	85	648
1005	1024	524738	GCAGCAAAACCCAAAAGACC	70	649
1025	1044	524739	AACCACATTGTGTAAATGGG	90	650
1028	1047	524740	GATAACCACATTGTGTAAAT	58	651
1031	1050	524741	CAGGATAACCACATTGTGTA	83	652
1034	1053	524742	ACGCAGGATAACCACATTGT	84	653
1037	1056	524743	TTAACGCAGGATAACCACAT	93	654
1040	1059	524744	GCATTAACGCAGGATAACCA	60	655
1043	1062	524745	AGGGCATTAACGCAGGATAA	58	656
1046	1065	524746	ACAAGGGCATTAACGCAGGA	75	657
1049	1068	524747	CATACAAGGGCATTAACGCA	89	658
1052	1071	524748	ATGCATACAAGGGCATTAAC	87	659
1055	1074	524749	TACATGCATACAAGGGCATT	86	660
1058	1077	524750	GAATACATGCATACAAGGGC	75	661
1061	1080	524751	ATTGAATACATGCATACAAG	81	662
1064	1083	524752	TAGATTGAATACATGCATAC	85	663

1067	1086	524753	GCTTAGATTGAATACATGCA	69	664
1070	1089	524754	CCTGCTTAGATTGAATACAT	90	665
1073	1092	524755	AAGCCTGCTTAGATTGAATA	76	666
1076	1095	524756	TGAAAGCCTGCTTAGATTGA	76	667
1079	1098	524757	AAGTGAAAGCCTGCTTAGAT	68	668
1082	1101	524758	AGAAAGTGAAAGCCTGCTTA	81	669
1085	1104	524759	GCGAGAAAGTGAAAGCCTGC	61	670
1088	1107	524760	TTGGCGAGAAAGTGAAAGCC	89	671
1091	1110	524761	AAGTTGGCGAGAAAGTGAAA	74	672
1094	1113	524762	TGTAAGTTGGCGAGAAAGTG	85	673
1097	1116	524763	CCTTGTAAGTTGGCGAGAAA	90	674
1100	1119	524764	AGGCCTTGTAAGTTGGCGAG	93	675
1103	1122	524765	GAAAGGCCTTGTAAGTTGGC	78	676
1106	1125	524766	ACAGAAAGGCCTTGTAAGTT	76	677
1109	1128	524767	TACACAGAAAGGCCTTGTAA	94	678
1112	1131	524768	GTTTACACAGAAAGGCCTTG	80	679
1115	1134	524769	ATTGTTTACACAGAAAGGCC	83	680
1118	1137	524770	GGTATTGTTTACACAGAAAG	63	681
1121	1140	524771	TCAGGTATTGTTTACACAGA	93	682
1124	1143	524772	GGTTCAGGTATTGTTTACAC	68	683
1127	1146	524773	AAAGGTTCAGGTATTGTTTA	82	684
1130	1149	524774	GGTAAAGGTTCAGGTATTGT	68	685
1150	1169	524775	TGGCCGTTGCCGGGCAACGG	74	686
1153	1172	524776	ACCTGGCCGTTGCCGGGCAA	77	687
1156	1175	524777	CAGACCTGGCCGTTGCCGGG	88	688
1159	1178	524778	GCACAGACCTGGCCGTTGCC	80	689
1162	1181	524779	TTGGCACAGACCTGGCCGTT	85	690
1165	1184	524780	CACTTGGCACAGACCTGGCC	93	691
1168	1187	524781	AAACACTTGGCACAGACCTG	90	692
1169	1188	524782	CAAACACTTGGCACAGACCT	75	693
1170	1189	505345	GCAAACACTTGGCACAGACC	78	207
1171	1190	524783	AGCAAACACTTGGCACAGAC	84	694
1172	1191	524784	CAGCAAACACTTGGCACAGA	90	695
1174	1193	524785	GTCAGCAAACACTTGGCACA	79	696
1200	1219	524786	ACCAAGCCCCAGCCAGTGGG	57	697
1203	1222	524787	ATGACCAAGCCCCAGCCAGT	74	698
1206	1225	524788	CCCATGACCAAGCCCCAGCC	90	699
1209	1228	524789	TGGCCCATGACCAAGCCCCA	96	700
1212	1231	524790	TGATGGCCCATGACCAAGCC	79	701
1215	1234	524791	CGCTGATGGCCCATGACCAA	97	702
1218	1237	524792	ACGCGCTGATGGCCCATGAC	98	703
1221	1240	524793	CGCACGCGCTGATGGCCCAT	98	704
1224	1243	524794	CCACGCACGCGCTGATGGCC	98	705

1227	1246	524795	GTTCCACGCACGCGCTGATG	98	706
1230	1249	524796	AAGGTTCCACGCACGCGCTG	99	707
1233	1252	524797	GAAAAGGTTCCACGCACGCG	97	708
1236	1255	524798	GCCGAAAAGGTTCCACGCAC	98	709
1239	1258	524799	GGAGCCGAAAAGGTTCCACG	75	710
1242	1261	524800	AGAGGAGCCGAAAAGGTTCC	79	711
1245	1264	524801	GGCAGAGGAGCCGAAAAGGT	98	712
1248	1267	524802	ATCGGCAGAGGAGCCGAAAA	73	713
1251	1270	524803	TGGATCGGCAGAGGAGCCGA	91	714
1254	1273	524804	GTATGGATCGGCAGAGGAGC	98	715
1257	1276	524805	GCAGTATGGATCGGCAGAGG	98	716
1258	1277	524806	CGCAGTATGGATCGGCAGAG	98	717
1259	1278	505346	CCGCAGTATGGATCGGCAGA	98	210
1260	1279	146785	TCCGCAGTATGGATCGGCAG	98	718
1261	1280	524807	TTCCGCAGTATGGATCGGCA	98	719
1262	1281	505347	GTTCCGCAGTATGGATCGGC	98	212
1263	1282	524808	AGTTCCGCAGTATGGATCGG	96	720
1264	1283	524809	GAGTTCCGCAGTATGGATCG	97	721
1266	1285	524810	AGGAGTTCCGCAGTATGGAT	96	722
1269	1288	524811	GCTAGGAGTTCCGCAGTATG	96	723
1272	1291	524812	GCGGCTAGGAGTTCCGCAGT	75	724
1275	1294	524813	CAAGCGGCTAGGAGTTCCGC	86	725
1278	1297	524814	AAACAAGCGGCTAGGAGTTC	73	726
1281	1300	524815	GCAAAACAAGCGGCTAGGAG	71	727
1282	1301	524816	AGCAAAACAAGCGGCTAGGA	89	728
1283	1302	505352	GAGCAAAACAAGCGGCTAGG	76	217
1284	1303	524817	CGAGCAAAACAAGCGGCTAG	78	729
1285	1304	524818	GCGAGCAAAACAAGCGGCTA	71	730
1286	1305	505353	TGCGAGCAAAACAAGCGGCT	82	218
1287	1306	524819	CTGCGAGCAAAACAAGCGGC	82	731
1288	1307	524820	GCTGCGAGCAAAACAAGCGG	67	732
1290	1309	524821	CTGCTGCGAGCAAAACAAGC	79	733
1293	1312	524822	GACCTGCTGCGAGCAAAACA	87	734
1296	1315	524823	CCAGACCTGCTGCGAGCAAA	94	735
1299	1318	524824	GCTCCAGACCTGCTGCGAGC	80	736
1302	1321	524825	TTTGCTCCAGACCTGCTGCG	70	737
1305	1324	524826	ATGTTTGCTCCAGACCTGCT	75	738
1308	1327	524827	ATAATGTTTGCTCCAGACCT	55	739
1311	1330	524828	CCGATAATGTTTGCTCCAGA	87	740
1314	1333	524829	GTCCCGATAATGTTTGCTCC	80	741
1317	1336	524830	TCAGTCCCGATAATGTTTGC	76	742
1320	1339	524831	TTATCAGTCCCGATAATGTT	53	743
1577	1596	146786	GTGAAGCGAAGTGCACACGG	96	224

Example 11: Antisense inhibition of HBV viral mRNA in HepG2.2.15 cells by MOE gapmers

Additional antisense oligonucleotides were designed targeting a HBV viral nucleic acid and were tested for their effects on HBV mRNA in vitro. Several of the antisense oligonucleotides from the studies described above were also included in the assay. Cultured HepG2.2.15 cells at a density of 28,000 cells per well were transfected using LipofectAMINE 2000® reagent with 70 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe set RTS3370 was used to measure mRNA levels. The mRNA levels of some of the gapmers were also measured using RTS3372. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in Tables 24 and 25 were designed as 5-10-5 MOE gapmers. The gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has an MOE sugar modification. Each nucleoside in the central gap segment has a deoxy sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines.

"Viral Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the viral gene sequence. "Viral Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted viral gene sequence. Each gapmer listed in Table 24 is targeted to the viral genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. U95551.1). Each gapmer listed in Table 25 is targeted to the viral genomic sequence, designated herein as SEQ ID NO: 1286 (a permuted version of GENBANK Accession No. U95551.1). 'n/a' indicates that the inhibition data for that particular gapmer was not measured with that particular primer probe set.

Table 24

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1 (RTS3370 and RTS3372)

Start Site	Stop Site	ISIS No	Sequence	RTS3370 % inhibition	RTS3372 % inhibition	SEQ ID NO
1323	1342	524832	GAGTTATCAGTCCCGATAAT	63	n/a	744
1326	1345	524833	ACAGAGTTATCAGTCCCGAT	82	n/a	745
1329	1348	524834	ACAACAGAGTTATCAGTCCC	52	n/a	746
1332	1351	524835	AGGACAACAGAGTTATCAGT	57	n/a	747
1335	1354	524836	GAGAGGACAACAGAGTTATC	49	n/a	748

1338	1357	524837	CGGGAGAGGACAACAGAGTT	0	n/a	749
1341	1360	524838	TTGCGGGAGAGGACAACAGA	17	n/a	750
1344	1363	524839	TATTTGCGGGAGAGGACAAC	30	n/a	751
1347	1366	524840	GTATATTTGCGGGAGAGGAC	22	n/a	752
1350	1369	524841	GATGTATATTTGCGGGAGAG	32	n/a	753
1353	1372	524842	TACGATGTATATTTGCGGGA	76	n/a	754
1356	1375	524843	GGATACGATGTATATTTGCG	76	n/a	755
1359	1378	524844	CATGGATACGATGTATATTT	87	n/a	756
1362	1381	524845	AGCCATGGATACGATGTATA	70	n/a	757
1365	1384	524846	AGCAGCCATGGATACGATGT	22	n/a	758
1368	1387	524847	CCTAGCAGCCATGGATACGA	67	n/a	759
1371	1390	524848	CAGCCTAGCAGCCATGGATA	56	n/a	760
1374	1393	524849	GCACAGCCTAGCAGCCATGG	38	n/a	761
1377	1396	524850	GCAGCACAGCCTAGCAGCCA	11	n/a	762
1380	1399	524851	TTGGCAGCACAGCCTAGCAG	34	n/a	763
1383	1402	524852	CAGTTGGCAGCACAGCCTAG	47	n/a	764
1386	1405	524853	ATCCAGTTGGCAGCACAGCC	45	n/a	765
1389	1408	524854	AGGATCCAGTTGGCAGCACA	36	n/a	766
1392	1411	524855	CGCAGGATCCAGTTGGCAGC	41	n/a	767
1395	1414	524856	CCGCGCAGGATCCAGTTGGC	72	n/a	768
1398	1417	524857	GTCCCGCGCAGGATCCAGTT	55	n/a	769
1457	1476	524858	AGCGACCCCGAGAAGGGTCG	17	n/a	770
1460	1479	524859	CCAAGCGACCCCGAGAAGGG	45	n/a	771
1463	1482	524860	GTCCCAAGCGACCCCGAGAA	8	n/a	772
1466	1485	524861	AGAGTCCCAAGCGACCCCGA	51	n/a	773
1469	1488	524862	GAGAGAGTCCCAAGCGACCC	28	n/a	774
1472	1491	524863	GACGAGAGAGTCCCAAGCGA	37	n/a	775
1492	1511	524864	GAACGGCAGACGGAGAAGGG	27	n/a	776
1498	1517	524866	CGGTCGGAACGGCAGACGGA	78	n/a	777
1501	1520	524867	GGTCGGTCGGAACGGCAGAC	78	n/a	778
1504	1523	524868	CGTGGTCGGTCGGAACGGCA	79	n/a	779
1507	1526	524869	CCCCGTGGTCGGTCGGAACG	70	n/a	780
1510	1529	524870	GCGCCCCGTGGTCGGTCGGA	78	n/a	781
1513	1532	524871	GGTGCGCCCCGTGGTCGGTC	74	n/a	782
1514	1533	524872	AGGTGCGCCCCGTGGTCGGT	63	n/a	783
1515	1534	505354	GAGGTGCGCCCCGTGGTCGG	70	n/a	220
1516	1535	524873	AGAGGTGCGCCCCGTGGTCG	72	n/a	784
1517	1536	524874	GAGAGGTGCGCCCCGTGGTC	49	n/a	785
1518	1537	505355	AGAGAGGTGCGCCCCGTGGT	64	n/a	221
1519	1538	524875	AAGAGAGGTGCGCCCCGTGG	57	n/a	786
1520	1539	524876	AAAGAGAGGTGCGCCCCGTG	63	n/a	787
1521	1540	505356	TAAAGAGAGGTGCGCCCCGT	68	n/a	222
1522	1541	524877	GTAAAGAGAGGTGCGCCCCG	50	n/a	788

1523	1542	524878	CGTAAAGAGAGGTGCGCCCC	64	n/a	789
1550	1569	524879	GATGAGAAGGCACAGACGGG	70	n/a	790
1553	1572	524880	GCAGATGAGAAGGCACAGAC	81	n/a	791
1556	1575	524881	CCGGCAGATGAGAAGGCACA	80	n/a	792
1559	1578	524882	GGTCCGGCAGATGAGAAGGC	84	n/a	793
1562	1581	524883	CACGGTCCGGCAGATGAGAA	79	n/a	794
1565	1584	524884	GCACACGGTCCGGCAGATGA	83	n/a	795
1568	1587	524885	AGTGCACACGGTCCGGCAGA	77	n/a	796
1571	1590	524886	CGAAGTGCACACGGTCCGGC	89	n/a	797
1574	1593	524887	AAGCGAAGTGCACACGGTCC	85	n/a	798
1575	1594	524888	GAAGCGAAGTGCACACGGTC	83	n/a	799
1576	1595	524889	TGAAGCGAAGTGCACACGGT	83	n/a	800
1577	1596	146786	GTGAAGCGAAGTGCACACGG	88	85	224
1578	1597	524890	GGTGAAGCGAAGTGCACACG	83	n/a	801
1579	1598	524891	AGGTGAAGCGAAGTGCACAC	82	n/a	802
1580	1599	505357	GAGGTGAAGCGAAGTGCACA	79	n/a	803
1581	1600	524892	AGAGGTGAAGCGAAGTGCAC	73	n/a	804
1582	1601	524893	CAGAGGTGAAGCGAAGTGCA	80	n/a	805
1583	1602	505358	GCAGAGGTGAAGCGAAGTGC	84	n/a	226
1584	1603	524894	TGCAGAGGTGAAGCGAAGTG	74	n/a	806
1585	1604	524895	GTGCAGAGGTGAAGCGAAGT	72	n/a	807
1586	1605	505359	CGTGCAGAGGTGAAGCGAAG	78	n/a	227
1604	1623	524896	ACGGTGGTCTCCATGCGACG	79	n/a	808
1607	1626	524897	TTCACGGTGGTCTCCATGCG	75	n/a	809
1630	1649	524898	CCTTGGGCAACATTCGGTGG	77	n/a	810
1633	1652	524899	AGACCTTGGGCAACATTCGG	76	n/a	811
1636	1655	524900	GTAAGACCTTGGGCAACATT	73	n/a	812
1639	1658	524901	TATGTAAGACCTTGGGCAAC	60	n/a	813
1642	1661	524902	TCTTATGTAAGACCTTGGGC	72	n/a	814
1645	1664	524903	TCCTCTTATGTAAGACCTTG	75	n/a	815
1648	1667	524904	GAGTCCTCTTATGTAAGACC	65	n/a	816
1651	1670	524905	CAAGAGTCCTCTTATGTAAG	76	n/a	817
1654	1673	524906	GTCCAAGAGTCCTCTTATGT	78	n/a	818
1657	1676	524907	AGAGTCCAAGAGTCCTCTTA	82	n/a	819
1660	1679	524908	CAGAGAGTCCAAGAGTCCTC	82	n/a	820
1663	1682	524909	TTGCAGAGAGTCCAAGAGTC	76	n/a	821
1666	1685	524910	ACATTGCAGAGAGTCCAAGA	76	n/a	822
1669	1688	524911	TTGACATTGCAGAGAGTCCA	74	n/a	823
1689	1708	524912	GTATGCCTCAAGGTCGGTCG	76	n/a	824
1692	1711	524913	GAAGTATGCCTCAAGGTCGG	73	n/a	825
1695	1714	524914	TTTGAAGTATGCCTCAAGGT	76	n/a	826
1698	1717	524915	GTCTTTGAAGTATGCCTCAA	75	n/a	827
1701	1720	524916	ACAGTCTTTGAAGTATGCCT	77	n/a	828

1704	1723	524917	CAAACAGTCTTTGAAGTATG	55	n/a	829
1707	1726	524918	AAACAAACAGTCTTTGAAGT	59	n/a	830
1710	1729	524919	TTTAAACAAACAGTCTTTGA	53	n/a	831
1713	1732	524920	GTCTTTAAACAAACAGTCTT	3	n/a	832
1716	1735	524921	CCAGTCTTTAAACAAACAGT	75	n/a	833
1719	1738	524922	CTCCCAGTCTTTAAACAAAC	70	n/a	834
1722	1741	524923	CTCCTCCCAGTCTTTAAACA	68	n/a	835
1725	1744	524924	CAACTCCTCCCAGTCTTTAA	62	n/a	836
1728	1747	524925	CCCCAACTCCTCCCAGTCTT	63	n/a	837
1731	1750	524926	CTCCCCCAACTCCTCCCAGT	62	n/a	838
1734	1753	524927	CTCCTCCCCCAACTCCTCCC	55	n/a	839
1737	1756	524928	AATCTCCTCCCCCAACTCCT	61	n/a	840
1740	1759	524929	TCTAATCTCCTCCCCCAACT	61	n/a	841
1743	1762	524930	TAATCTAATCTCCTCCCCCA	70	n/a	842
1746	1765	524931	CTTTAATCTAATCTCCTCCC	74	n/a	843
1749	1768	524932	GACCTTTAATCTAATCTCCT	74	n/a	844
1752	1771	524933	AAAGACCTTTAATCTAATCT	60	n/a	845
1755	1774	524934	TACAAAGACCTTTAATCTAA	55	n/a	846
1758	1777	524935	TAGTACAAAGACCTTTAATC	54	n/a	847
1761	1780	524936	TCCTAGTACAAAGACCTTTA	69	n/a	848
1764	1783	524937	GCCTCCTAGTACAAAGACCT	72	n/a	849
1767	1786	524938	ACAGCCTCCTAGTACAAAGA	60	n/a	850
1770	1789	524939	CCTACAGCCTCCTAGTACAA	66	n/a	851
1773	1792	524940	ATGCCTACAGCCTCCTAGTA	70	n/a	852
1776	1795	524941	TTTATGCCTACAGCCTCCTA	63	n/a	853
1777	1796	524942	ATTTATGCCTACAGCCTCCT	70	n/a	854
1778	1797	509932	AATTTATGCCTACAGCCTCC	68	n/a	46
1779	1798	509933	CAATTTATGCCTACAGCCTC	68	n/a	48
1780	1799	509934	CCAATTTATGCCTACAGCCT	65	n/a	50
1781	1800	509935	ACCAATTTATGCCTACAGCC	64	n/a	52
1782	1801	524943	GACCAATTTATGCCTACAGC	57	n/a	855
1783	1802	524944	AGACCAATTTATGCCTACAG	60	n/a	856
1785	1804	524945	GCAGACCAATTTATGCCTAC	54	n/a	857
1788	1807	524946	TGCGCAGACCAATTTATGCC	68	n/a	858
1791	1810	524947	TGGTGCGCAGACCAATTTAT	64	n/a	859
1794	1813	524948	TGCTGGTGCGCAGACCAATT	75	n/a	860
1797	1816	524949	TGGTGCTGGTGCGCAGACCA	68	n/a	861
1800	1819	524950	GCATGGTGCTGGTGCGCAGA	69	n/a	862
1803	1822	524951	GTTGCATGGTGCTGGTGCGC	59	n/a	863
1807	1826	524952	AAAAGTTGCATGGTGCTGGT	61	n/a	864
1810	1829	524953	TGAAAAAGTTGCATGGTGCT	60	n/a	865
1813	1832	524954	AGGTGAAAAAGTTGCATGGT	61	n/a	866
1816	1835	524955	CAGAGGTGAAAAAGTTGCAT	63	n/a	867

1819	1838	524956	AGGCAGAGGTGAAAAAGTTG	57	n/a	868
1822	1841	524957	ATTAGGCAGAGGTGAAAAAG	50	n/a	869
1823	1842	524958	GATTAGGCAGAGGTGAAAAA	57	n/a	870
1825	1844	524959	ATGATTAGGCAGAGGTGAAA	54	n/a	871
1828	1847	524960	GAGATGATTAGGCAGAGGTG	59	n/a	872
1831	1850	524961	CAAGAGATGATTAGGCAGAG	61	n/a	873
1834	1853	524962	GAACAAGAGATGATTAGGCA	56	n/a	874
1837	1856	524963	CATGAACAAGAGATGATTAG	24	n/a	875
1840	1859	524964	GGACATGAACAAGAGATGAT	54	n/a	876
1843	1862	524965	GTAGGACATGAACAAGAGAT	52	n/a	877
1846	1865	524966	ACAGTAGGACATGAACAAGA	47	n/a	878
1849	1868	524967	TGAACAGTAGGACATGAACA	33	n/a	879
1852	1871	524968	GCTTGAACAGTAGGACATGA	44	n/a	880
1855	1874	524969	GAGGCTTGAACAGTAGGACA	43	n/a	881
1858	1877	524970	TTGGAGGCTTGAACAGTAGG	28	n/a	882
1862	1881	524971	CAGCTTGGAGGCTTGAACAG	30	n/a	883
1871	1890	524972	CCCAAGGCACAGCTTGGAGG	38	n/a	884
1874	1893	524973	CCACCCAAGGCACAGCTTGG	47	n/a	885
1877	1896	524974	AAGCCACCCAAGGCACAGCT	49	n/a	886
1880	1899	524975	CCAAAGCCACCCAAGGCACA	32	n/a	887
1883	1902	524976	GCCCCAAAGCCACCCAAGGC	56	n/a	888
1886	1905	524977	CATGCCCCAAAGCCACCCAA	63	n/a	889
1889	1908	524978	GTCCATGCCCCAAAGCCACC	64	n/a	890
1892	1911	524979	GATGTCCATGCCCCAAAGCC	65	n/a	891
1895	1914	524980	GTCGATGTCCATGCCCCAAA	80	n/a	892
1898	1917	524981	AGGGTCGATGTCCATGCCCC	79	n/a	893
1901	1920	524982	ATAAGGGTCGATGTCCATGC	79	n/a	894
1904	1923	524983	TTTATAAGGGTCGATGTCCA	71	n/a	895
1907	1926	524984	TTCTTTATAAGGGTCGATGT	77	n/a	896
1910	1929	524985	AAATTCTTTATAAGGGTCGA	79	n/a	897
1913	1932	524986	TCCAAATTCTTTATAAGGGT	80	n/a	898
1916	1935	524987	AGCTCCAAATTCTTTATAAG	80	n/a	899
1919	1938	524988	AGTAGCTCCAAATTCTTTAT	76	n/a	900
1922	1941	524989	CACAGTAGCTCCAAATTCTT	59	n/a	901
1925	1944	524990	CTCCACAGTAGCTCCAAATT	46	n/a	902
1928	1947	524991	TAACTCCACAGTAGCTCCAA	63	n/a	903
1931	1950	524992	GAGTAACTCCACAGTAGCTC	65	n/a	904
1934	1953	524993	CGAGAGTAACTCCACAGTAG	69	n/a	905
1937	1956	524994	AAACGAGAGTAACTCCACAG	61	n/a	906
1940	1959	524995	CAAAAACGAGAGTAACTCCA	46	n/a	907
1943	1962	524996	AGGCAAAAACGAGAGTAACT	39	n/a	908
1946	1965	524997	AGAAGGCAAAAACGAGAGTA	53	n/a	909
1949	1968	524998	GTCAGAAGGCAAAAACGAGA	56	n/a	910

1952	1971	524999	GAAGTCAGAAGGCAAAAACG	49	n/a	911
1955	1974	525000	AAAGAAGTCAGAAGGCAAAA	29	n/a	912
1958	1977	525001	AGGAAAGAAGTCAGAAGGCA	41	n/a	913
1961	1980	525002	TGAAGGAAAGAAGTCAGAAG	34	n/a	914
1964	1983	525003	TACTGAAGGAAAGAAGTCAG	26	n/a	915
1984	2003	525004	GCGGTATCTAGAAGATCTCG	24	n/a	916
1987	2006	525005	GAGGCGGTATCTAGAAGATC	29	n/a	917
1990	2009	525006	GCTGAGGCGGTATCTAGAAG	29	n/a	918
1993	2012	525007	AGAGCTGAGGCGGTATCTAG	13	n/a	919
1996	2015	525008	TACAGAGCTGAGGCGGTATC	6	n/a	920
1999	2018	525009	CGATACAGAGCTGAGGCGGT	3	n/a	921
2002	2021	525010	TCCCGATACAGAGCTGAGGC	27	n/a	922
2005	2024	525011	GCTTCCCGATACAGAGCTGA	43	n/a	923
2008	2027	525012	AAGGCTTCCCGATACAGAGC	33	n/a	924
2011	2030	525013	TCTAAGGCTTCCCGATACAG	34	n/a	925
2014	2033	525014	GACTCTAAGGCTTCCCGATA	38	n/a	926
2017	2036	525015	GGAGACTCTAAGGCTTCCCG	16	n/a	927
2020	2039	525016	TCAGGAGACTCTAAGGCTTC	16	n/a	928
2023	2042	525017	TGCTCAGGAGACTCTAAGGC	14	n/a	929
2026	2045	525018	CAATGCTCAGGAGACTCTAA	34	n/a	930
2029	2048	525019	GAACAATGCTCAGGAGACTC	32	n/a	931
2032	2051	525020	GGTGAACAATGCTCAGGAGA	9	n/a	932
2035	2054	525021	TGAGGTGAACAATGCTCAGG	50	n/a	933
2038	2057	525022	TGGTGAGGTGAACAATGCTC	54	n/a	934
2041	2060	525023	GTATGGTGAGGTGAACAATG	47	n/a	935
2044	2063	525024	GCAGTATGGTGAGGTGAACA	40	n/a	936
2047	2066	525025	AGTGCAGTATGGTGAGGTGA	35	n/a	937
2050	2069	525026	CTGAGTGCAGTATGGTGAGG	43	n/a	938
2053	2072	525027	TGCCTGAGTGCAGTATGGTG	45	n/a	939
2056	2075	525028	GCTTGCCTGAGTGCAGTATG	42	n/a	940
2059	2078	525029	ATTGCTTGCCTGAGTGCAGT	39	n/a	941
2062	2081	525030	AGAATTGCTTGCCTGAGTGC	27	n/a	942
2065	2084	525031	CAAAGAATTGCTTGCCTGAG	42	n/a	943
2068	2087	525032	CAGCAAAGAATTGCTTGCCT	49	n/a	944
2071	2090	525033	CCCCAGCAAAGAATTGCTTG	41	n/a	945
2074	2093	525034	TCCCCCCAGCAAAGAATTGC	39	n/a	946
2077	2096	525035	AGTTCCCCCCAGCAAAGAAT	39	n/a	947
2080	2099	525036	ATTAGTTCCCCCCAGCAAAG	43	n/a	948
2083	2102	525037	GTCATTAGTTCCCCCCAGCA	64	n/a	949
2086	2105	525038	AGAGTCATTAGTTCCCCCCA	45	n/a	950
2089	2108	525039	GCTAGAGTCATTAGTTCCCC	58	n/a	951
2092	2111	525040	GTAGCTAGAGTCATTAGTTC	45	n/a	952
2095	2114	525041	CAGGTAGCTAGAGTCATTAG	44	n/a	953

2098	2117	525042	ACCCAGGTAGCTAGAGTCAT	39	n/a	954
2101	2120	525043	CCCACCCAGGTAGCTAGAGT	51	n/a	955
2104	2123	525044	ACACCCACCCAGGTAGCTAG	27	n/a	956
2107	2126	525045	TTAACACCCACCCAGGTAGC	41	n/a	957
2110	2129	525046	AAATTAACACCCACCCAGGT	44	n/a	958
2113	2132	525047	TCCAAATTAACACCCACCCA	29	n/a	959
2116	2135	525048	TCTTCCAAATTAACACCCAC	31	n/a	960
2119	2138	525049	GGATCTTCCAAATTAACACC	42	n/a	961
2122	2141	525050	GCTGGATCTTCCAAATTAAC	53	n/a	962
2125	2144	525051	GATGCTGGATCTTCCAAATT	41	n/a	963
2128	2147	525052	CTAGATGCTGGATCTTCCAA	62	n/a	964
2131	2150	525053	TCTCTAGATGCTGGATCTTC	41	83	965
2134	2153	525054	AGGTCTCTAGATGCTGGATC	26	73	966
2137	2156	525055	ACTAGGTCTCTAGATGCTGG	36	74	967
2140	2159	525056	ACTACTAGGTCTCTAGATGC	22	63	968
2143	2162	525057	CTGACTACTAGGTCTCTAGA	28	80	969
2146	2165	525058	TAACTGACTACTAGGTCTCT	47	83	970
2149	2168	525059	ACATAACTGACTACTAGGTC	31	77	971
2152	2171	525060	TTGACATAACTGACTACTAG	34	75	972
2155	2174	525061	GTGTTGACATAACTGACTAC	42	75	973
2158	2177	525062	TTAGTGTTGACATAACTGAC	48	81	974
2161	2180	525063	ATATTAGTGTTGACATAACT	33	73	975
2164	2183	525064	CCCATATTAGTGTTGACATA	41	82	976
2167	2186	525065	AGGCCCATATTAGTGTTGAC	39	77	977
2170	2189	525066	TTTAGGCCCATATTAGTGTT	46	83	978
2173	2192	525067	AACTTTAGGCCCATATTAGT	38	69	979
2176	2195	525068	CTGAACTTTAGGCCCATATT	41	85	980
2179	2198	525069	TGCCTGAACTTTAGGCCCAT	38	81	981
2182	2201	525070	AGTTGCCTGAACTTTAGGCC	17	67	982
2185	2204	525071	AAGAGTTGCCTGAACTTTAG	27	62	983
2188	2207	525072	CACAAGAGTTGCCTGAACTT	27	64	984
2191	2210	525073	AACCACAAGAGTTGCCTGAA	41	80	985
2194	2213	525074	TGAAACCACAAGAGTTGCCT	32	75	986
2197	2216	525075	ATGTGAAACCACAAGAGTTG	43	67	987
2200	2219	525076	GAAATGTGAAACCACAAGAG	34	74	988
2203	2222	525077	CAAGAAATGTGAAACCACAA	22	65	989
2206	2225	525078	AGACAAGAAATGTGAAACCA	39	70	990
2209	2228	525079	GTGAGACAAGAAATGTGAAA	32	74	991
2212	2231	525080	AAAGTGAGACAAGAAATGTG	30	63	992
2215	2234	525081	CCAAAAGTGAGACAAGAAAT	25	58	993
2218	2237	525082	CTTCCAAAAGTGAGACAAGA	36	74	994
2221	2240	525083	TCTCTTCCAAAAGTGAGACA	42	84	995
2224	2243	525084	GTTTCTCTTCCAAAAGTGAG	33	75	996

2227	2246	525085	ACGGTTTCTCTTCCAAAAGT	32	68	997
2230	2249	525086	ATAACGGTTTCTCTTCCAAA	51	80	998
2233	2252	525087	TCTATAACGGTTTCTCTTCC	36	77	999
2236	2255	525088	TACTCTATAACGGTTTCTCT	23	69	1000
2239	2258	525089	AAATACTCTATAACGGTTTC	45	77	1001
2242	2261	525090	ACCAAATACTCTATAACGGT	57	82	1002
2245	2264	525091	GACACCAAATACTCTATAAC	36	77	1003
2248	2267	525092	AAAGACACCAAATACTCTAT	42	80	1004
2251	2270	525093	CCGAAAGACACCAAATACTC	41	89	1005
2254	2273	525094	ACTCCGAAAGACACCAAATA	29	73	1006
2257	2276	525095	CACACTCCGAAAGACACCAA	33	92	1007
2260	2279	525096	ATCCACACTCCGAAAGACAC	18	74	1008
2263	2282	525097	CGAATCCACACTCCGAAAGA	30	57	1009
2266	2285	525098	GTGCGAATCCACACTCCGAA	28	67	1010
2269	2288	146789	GGAGTGCGAATCCACACTCC	37	72	1011
2272	2291	525099	GGAGGAGTGCGAATCCACAC	36	64	1012
2275	2294	525100	GCTGGAGGAGTGCGAATCCA	52	90	1013
2278	2297	525101	TAAGCTGGAGGAGTGCGAAT	49	96	1014
2281	2300	525102	CTATAAGCTGGAGGAGTGCG	37	96	1015
2284	2303	525103	GGTCTATAAGCTGGAGGAGT	30	97	1016
2287	2306	525104	GGTGGTCTATAAGCTGGAGG	22	77	1017
2290	2309	525105	TTTGGTGGTCTATAAGCTGG	41	76	1018
2293	2312	525106	GCATTTGGTGGTCTATAAGC	39	76	1019
2313	2332	525107	GAAGTGTTGATAGGATAGGG	27	97	1020
2316	2335	525108	CCGGAAGTGTTGATAGGATA	42	97	1021
2319	2338	525109	TTTCCGGAAGTGTTGATAGG	48	99	1022
2322	2341	525110	TAGTTTCCGGAAGTGTTGAT	18	98	1023
2325	2344	525111	CAGTAGTTTCCGGAAGTGTT	19	98	1024
2328	2347	525112	CAACAGTAGTTTCCGGAAGT	29	96	1025
2331	2350	525113	TAACAACAGTAGTTTCCGGA	39	95	1026
2334	2353	525114	GTCTAACAACAGTAGTTTCC	40	99	1027
2369	2388	525115	CGAGGGAGTTCTTCTTCTAG	42	98	1028
2372	2391	525116	AGGCGAGGGAGTTCTTCTTC	31	97	1029
2375	2394	525117	GCGAGGCGAGGGAGTTCTTC	22	98	1030
2379	2398	525118	GTCTGCGAGGCGAGGGAGTT	20	99	1031
2398	2417	525119	CGCGGCGATTGAGACCTTCG	26	97	1032
2401	2420	525120	CGACGCGGCGATTGAGACCT	23	97	1033
2404	2423	525121	CTGCGACGCGGCGATTGAGA	47	92	1034
2407	2426	525122	CTTCTGCGACGCGGCGATTG	27	74	1035
2410	2429	525123	GATCTTCTGCGACGCGGCGA	36	87	1036
2413	2432	146790	TGAGATCTTCTGCGACGCGG	25	85	1037
2416	2435	525124	GATTGAGATCTTCTGCGACG	17	84	1038
2419	2438	525125	CGAGATTGAGATCTTCTGCG	24	82	1039

2422	2441	525126	TCCCGAGATTGAGATCTTCT	29	74	1040
2425	2444	525127	GGTTCCCGAGATTGAGATCT	14	79	1041
2428	2447	525128	TGAGGTTCCCGAGATTGAGA	41	76	1042
2431	2450	525129	CATTGAGGTTCCCGAGATTG	39	72	1043
2434	2453	525130	TAACATTGAGGTTCCCGAGA	37	71	1044
2437	2456	525131	TACTAACATTGAGGTTCCCG	42	76	1045
2440	2459	525132	GAATACTAACATTGAGGTTC	21	75	1046
2443	2462	525133	AAGGAATACTAACATTGAGG	36	75	1047
2446	2465	525134	TCCAAGGAATACTAACATTG	29	77	1048
2449	2468	525135	GAGTCCAAGGAATACTAACA	32	76	1049
2452	2471	525136	TATGAGTCCAAGGAATACTA	23	62	1050
2455	2474	525137	CCTTATGAGTCCAAGGAATA	27	57	1051
2458	2477	525138	CCACCTTATGAGTCCAAGGA	52	82	1052
2461	2480	525139	TCCCCACCTTATGAGTCCAA	46	80	1053
2464	2483	525140	AGTTCCCCACCTTATGAGTC	14	59	1054
2467	2486	525141	TAAAGTTCCCCACCTTATGA	20	45	1055
2470	2489	525142	CAGTAAAGTTCCCCACCTTA	14	72	1056
2473	2492	525143	GACCAGTAAAGTTCCCCACC	30	77	1057
2476	2495	525144	AAAGACCAGTAAAGTTCCCC	19	72	1058
2479	2498	525145	AATAAAGACCAGTAAAGTTC	18	55	1059
2482	2501	525146	AAGAATAAAGACCAGTAAAG	16	51	1060
2485	2504	525147	TAGAAGAATAAAGACCAGTA	22	68	1061
2488	2507	525148	CAGTAGAAGAATAAAGACCA	13	59	1062
2491	2510	525149	GTACAGTAGAAGAATAAAGA	0	45	1063
2494	2513	525150	CAGGTACAGTAGAAGAATAA	31	62	1064
2497	2516	525151	AGACAGGTACAGTAGAAGAA	8	62	1065
2500	2519	525152	TAAAGACAGGTACAGTAGAA	29	61	1066
2503	2522	525153	GATTAAAGACAGGTACAGTA	28	67	1067
2506	2525	525154	GAGGATTAAAGACAGGTACA	38	76	1068
2509	2528	525155	AATGAGGATTAAAGACAGGT	30	72	1069
2512	2531	525156	TCCAATGAGGATTAAAGACA	24	67	1070
2515	2534	525157	TTTTCCAATGAGGATTAAAG	0	44	1071
2518	2537	525158	GTGTTTTCCAATGAGGATTA	20	74	1072
2521	2540	525159	ATGGTGTTTTCCAATGAGGA	30	71	1073
2524	2543	525160	AAGATGGTGTTTTCCAATGA	22	68	1074
2527	2546	525161	GAAAAGATGGTGTTTTCCAA	19	61	1075
2530	2549	525162	TAGGAAAAGATGGTGTTTTC	14	52	1076
2533	2552	525163	TATTAGGAAAAGATGGTGTT	1	47	1077
2536	2555	525164	GTATATTAGGAAAAGATGGT	0	60	1078
2539	2558	525165	AATGTATATTAGGAAAAGAT	0	30	1079
2542	2561	525166	GTAAATGTATATTAGGAAAA	1	18	1080
2545	2564	525167	GGTGTAAATGTATATTAGGA	23	72	1081
2548	2567	525168	CTTGGTGTAAATGTATATTA	32	75	1082

2551	2570	525169	TGTCTTGGTGTAAATGTATA	12	65	1083
2554	2573	525170	TAATGTCTTGGTGTAAATGT	3	51	1084
2557	2576	525171	TGATAATGTCTTGGTGTAAA	24	62	1085
2560	2579	525172	TTTTGATAATGTCTTGGTGT	18	66	1086
2563	2582	525173	ATTTTTTGATAATGTCTTGG	11	63	1087
2566	2585	525174	CACATTTTTTGATAATGTCT	20	68	1088
2569	2588	525175	GTTCACATTTTTTGATAATG	38	68	1089
2572	2591	525176	ACTGTTCACATTTTTTGATA	12	61	1090
2575	2594	525177	CAAACTGTTCACATTTTTTG	25	56	1091
2578	2597	525178	CTACAAACTGTTCACATTTT	21	47	1092
2581	2600	525179	GGCCTACAAACTGTTCACAT	28	83	1093
2584	2603	525180	GTGGGCCTACAAACTGTTCA	7	72	1094
2587	2606	525181	TAAGTGGGCCTACAAACTGT	26	75	1095
2590	2609	525182	CTGTAAGTGGGCCTACAAAC	35	78	1096
2593	2612	525183	TAACTGTAAGTGGGCCTACA	29	69	1097
2596	2615	525184	CATTAACTGTAAGTGGGCCT	22	73	1098
2599	2618	525185	TCTCATTAACTGTAAGTGGG	31	81	1099
2602	2621	525186	TTTTCTCATTAACTGTAAGT	15	58	1100
2605	2624	525187	TTCTTTTCTCATTAACTGTA	14	71	1101
2608	2627	525188	ATCTTCTTTTCTCATTAACT	19	71	1102
2611	2630	525189	GCAATCTTCTTTTCTCATTA	36	79	1103
2614	2633	525190	ATTGCAATCTTCTTTTCTCA	38	82	1104
2617	2636	525191	TCAATTGCAATCTTCTTTTC	23	61	1105
2620	2639	525192	TAATCAATTGCAATCTTCTT	10	67	1106
2623	2642	525193	GCATAATCAATTGCAATCTT	27	71	1107
2626	2645	525194	CAGGCATAATCAATTGCAAT	23	71	1108
2629	2648	525195	TAGCAGGCATAATCAATTGC	30	77	1109
2632	2651	525196	ACCTAGCAGGCATAATCAAT	7	70	1110
2635	2654	525197	AAAACCTAGCAGGCATAATC	47	70	1111
2638	2657	525198	GATAAAACCTAGCAGGCATA	41	81	1112
2641	2660	525199	TTGGATAAAACCTAGCAGGC	30	78	1113
2644	2663	525200	CCTTTGGATAAAACCTAGCA	31	76	1114
2647	2666	525201	TAACCTTTGGATAAAACCTA	25	63	1115
2650	2669	525202	TGGTAACCTTTGGATAAAAC	24	76	1116
2653	2672	525203	ATTTGGTAACCTTTGGATAA	20	64	1117
2656	2675	525204	AATATTTGGTAACCTTTGGA	16	77	1118
2659	2678	525205	GTAAATATTTGGTAACCTTT	39	80	1119
2662	2681	525206	ATGGTAAATATTTGGTAACC	40	75	1120
2665	2684	525207	CCAATGGTAAATATTTGGTA	38	75	1121
2668	2687	525208	TATCCAATGGTAAATATTTG	0	0	1122
2671	2690	525209	CCTTATCCAATGGTAAATAT	28	57	1123
2674	2693	525210	TACCCTTATCCAATGGTAAA	18	71	1124
2677	2696	525211	TAATACCCTTATCCAATGGT	35	76	1125

2680	2699	525212	GTTTAATACCCTTATCCAAT	41	77	1126
2683	2702	525213	AAGGTTTAATACCCTTATCC	11	79	1127
2686	2705	525214	AATAAGGTTTAATACCCTTA	35	75	1128
2689	2708	525215	GATAATAAGGTTTAATACCC	22	54	1129
2692	2711	525216	CTGGATAATAAGGTTTAATA	19	35	1130
2695	2714	525217	GTTCTGGATAATAAGGTTTA	24	58	1131
2698	2717	525218	GATGTTCTGGATAATAAGGT	20	73	1132
2701	2720	525219	CTAGATGTTCTGGATAATAA	26	66	1133
2704	2723	525220	TAACTAGATGTTCTGGATAA	21	66	1134
2707	2726	525221	GATTAACTAGATGTTCTGGA	30	78	1135
2710	2729	525222	AATGATTAACTAGATGTTCT	30	61	1136
2713	2732	525223	AGTAATGATTAACTAGATGT	9	57	1137
2716	2735	525224	GGAAGTAATGATTAACTAGA	18	72	1138
2719	2738	525225	TTTGGAAGTAATGATTAACT	7	67	1139
2722	2741	525226	TAGTTTGGAAGTAATGATTA	2	30	1140
2725	2744	525227	GTCTAGTTTGGAAGTAATGA	27	78	1141
2728	2747	525228	AGTGTCTAGTTTGGAAGTAA	27	75	1142
2731	2750	525229	AATAGTGTCTAGTTTGGAAG	34	73	1143
2734	2753	525230	GTAAATAGTGTCTAGTTTGG	28	68	1144
2737	2756	525231	TGTGTAAATAGTGTCTAGTT	27	79	1145
2740	2759	525232	GAGTGTGTAAATAGTGTCTA	27	71	1146
2743	2762	525233	ATAGAGTGTGTAAATAGTGT	17	75	1147
2746	2765	525234	TCCATAGAGTGTGTAAATAG	18	75	1148
2749	2768	525235	CCTTCCATAGAGTGTGTAAA	23	80	1149
2752	2771	525236	CCGCCTTCCATAGAGTGTGT	26	82	1150
2755	2774	525237	TACCCGCCTTCCATAGAGTG	19	80	1151
2758	2777	525238	ATATACCCGCCTTCCATAGA	0	67	1152
2761	2780	525239	ATAATATACCCGCCTTCCAT	19	70	1153
2764	2783	525240	TATATAATATACCCGCCTTC	9	73	1154
2767	2786	525241	TCTTATATAATATACCCGCC	20	80	1155
2770	2789	525242	CTCTCTTATATAATATACCC	29	76	1156
2773	2792	525243	TTTCTCTCTTATATAATATA	16	58	1157
2776	2795	525244	TTGTTTCTCTCTTATATAAT	26	57	1158
2779	2798	525245	GTGTTGTTTCTCTCTTATAT	35	85	1159
2782	2801	525246	TATGTGTTGTTTCTCTCTTA	34	82	1160
2785	2804	525247	CGCTATGTGTTGTTTCTCTC	34	86	1161
2802	2821	525248	TGACCCACAAAATGAGGCGC	17	71	1162
2805	2824	525249	TGGTGACCCACAAAATGAGG	31	67	1163
2808	2827	525250	ATATGGTGACCCACAAAATG	38	69	1164
2811	2830	525251	AGAATATGGTGACCCACAAA	37	77	1165
2814	2833	525252	CCAAGAATATGGTGACCCAC	35	79	1166
2817	2836	146831	TTCCCAAGAATATGGTGACC	27	75	1167
2820	2839	525253	TTGTTCCCAAGAATATGGTG	33	69	1168

2823	2842	525254	ATCTTGTTCCCAAGAATATG	27	65	1169
2826	2845	525255	TAGATCTTGTTCCCAAGAAT	31	70	1170
2829	2848	525256	CTGTAGATCTTGTTCCCAAG	42	81	1171
2832	2851	525257	ATGCTGTAGATCTTGTTCCC	34	80	1172
2835	2854	525258	CCCATGCTGTAGATCTTGTT	38	80	1173
2838	2857	525259	TGCCCCATGCTGTAGATCTT	36	80	1174
2841	2860	525260	TTCTGCCCCATGCTGTAGAT	32	74	1175
2844	2863	525261	AGATTCTGCCCCATGCTGTA	27	75	1176
2847	2866	525262	GAAAGATTCTGCCCCATGCT	34	70	1177
2850	2869	525263	GTGGAAAGATTCTGCCCCAT	22	76	1178
2853	2872	525264	CTGGTGGAAAGATTCTGCCC	36	72	1179
2856	2875	525265	TTGCTGGTGGAAAGATTCTG	32	71	1180
2859	2878	525266	GGATTGCTGGTGGAAAGATT	20	74	1181
2862	2881	525267	AGAGGATTGCTGGTGGAAAG	25	73	1182
2865	2884	525268	CCCAGAGGATTGCTGGTGGA	40	82	1183
2868	2887	525269	AATCCCAGAGGATTGCTGGT	32	79	1184
2871	2890	525270	AAGAATCCCAGAGGATTGCT	23	69	1185
2874	2893	525271	GGAAAGAATCCCAGAGGATT	10	66	1186
2877	2896	525272	TCGGGAAAGAATCCCAGAGG	29	73	1187
2880	2899	525273	TGGTCGGGAAAGAATCCCAG	31	77	1188
2883	2902	525274	TGGTGGTCGGGAAAGAATCC	38	71	1189
2886	2905	525275	AACTGGTGGTCGGGAAAGAA	33	78	1190
2889	2908	525276	TCCAACTGGTGGTCGGGAAA	29	76	1191
2892	2911	525277	GGATCCAACTGGTGGTCGGG	19	81	1192
2895	2914	525278	GCTGGATCCAACTGGTGGTC	24	74	1193
2898	2917	525279	AAGGCTGGATCCAACTGGTG	33	83	1194
2901	2920	525280	CTGAAGGCTGGATCCAACTG	18	81	1195
2904	2923	525286	GCTCTGAAGGCTGGATCCAA	40	79	1196
2907	2926	525287	TTTGCTCTGAAGGCTGGATC	34	69	1197
2910	2929	525288	GTGTTTGCTCTGAAGGCTGG	38	72	1198
2913	2932	525289	GCTGTGTTTGCTCTGAAGGC	40	82	1199
2916	2935	525290	TTTGCTGTGTTTGCTCTGAA	44	78	1200
2919	2938	525291	GGATTTGCTGTGTTTGCTCT	38	76	1201
2922	2941	525292	TCTGGATTTGCTGTGTTTGC	28	79	1202
2925	2944	525293	CAATCTGGATTTGCTGTGTT	26	61	1203
2928	2947	525294	TCCCAATCTGGATTTGCTGT	32	68	1204
2931	2950	525295	AAGTCCCAATCTGGATTTGC	33	59	1205
2934	2953	146832	TTGAAGTCCCAATCTGGATT	17	35	1206
2937	2956	525296	GGATTGAAGTCCCAATCTGG	35	62	1207
2940	2959	525297	TTGGGATTGAAGTCCCAATC	10	36	1208
2943	2962	525298	TTGTTGGGATTGAAGTCCCA	24	49	1209
2946	2965	525299	TCCTTGTTGGGATTGAAGTC	16	52	1210
2949	2968	525300	GTGTCCTTGTTGGGATTGAA	18	71	1211

2952	2971	525301	CAGGTGTCCTTGTTGGGATT	25	73	1212
2955	2974	525302	GGCCAGGTGTCCTTGTTGGG	31	70	1213
2958	2977	525303	TCTGGCCAGGTGTCCTTGTT	29	75	1214
2978	2997	525304	CAGCTCCTACCTTGTTGGCG	29	71	1215
2981	3000	525305	CTCCAGCTCCTACCTTGTTG	19	63	1216
2984	3003	525306	ATGCTCCAGCTCCTACCTTG	35	75	1217
2987	3006	525307	CGAATGCTCCAGCTCCTACC	13	77	1218
2990	3009	525308	GCCCGAATGCTCCAGCTCCT	28	72	1219
2993	3012	525309	CCAGCCCGAATGCTCCAGCT	32	77	1220
2996	3015	525310	AACCCAGCCCGAATGCTCCA	34	72	1221
2999	3018	525311	TGAAACCCAGCCCGAATGCT	28	69	1222
3002	3021	525312	GGGTGAAACCCAGCCCGAAT	18	68	1223
3020	3039	525313	AAAGGCCTCCGTGCGGTGGG	36	77	1224
3023	3042	525314	CCAAAAGGCCTCCGTGCGGT	34	83	1225
3026	3045	525315	ACCCCAAAAGGCCTCCGTGC	28	70	1226
3029	3048	525316	TCCACCCCAAAAGGCCTCCG	26	65	1227
3032	3051	525317	GGCTCCACCCCAAAAGGCCT	19	36	1228
3035	3054	525318	GAGGGCTCCACCCCAAAAGG	14	36	1229
3038	3057	525319	CCTGAGGGCTCCACCCCAAA	32	71	1230
3041	3060	525320	GAGCCTGAGGGCTCCACCCC	37	61	1231
3044	3063	525321	CCTGAGCCTGAGGGCTCCAC	42	70	1232
3047	3066	525322	TGCCCTGAGCCTGAGGGCTC	24	56	1233
3050	3069	525323	GTATGCCCTGAGCCTGAGGG	14	75	1234
3053	3072	525324	GTAGTATGCCCTGAGCCTGA	29	83	1235
3056	3075	525325	TTTGTAGTATGCCCTGAGCC	32	61	1236
3059	3078	525326	AAGTTTGTAGTATGCCCTGA	35	70	1237
3062	3081	525327	GCAAAGTTTGTAGTATGCCC	37	61	1238
3065	3084	525328	CTGGCAAAGTTTGTAGTATG	26	63	1239
3068	3087	525329	TTGCTGGCAAAGTTTGTAGT	37	74	1240
3071	3090	525330	GATTTGCTGGCAAAGTTTGT	20	56	1241
3074	3093	525331	GCGGATTTGCTGGCAAAGTT	28	80	1242
3077	3096	525332	GAGGCGGATTTGCTGGCAAA	38	74	1243
3080	3099	525333	CAGGAGGCGGATTTGCTGGC	41	66	1244
3083	3102	525334	AGGCAGGAGGCGGATTTGCT	27	55	1245
3086	3105	525335	TGGAGGCAGGAGGCGGATTT	13	17	1246
3089	3108	525336	TGGTGGAGGCAGGAGGCGGA	7	21	1247
3092	3111	525337	GATTGGTGGAGGCAGGAGGC	21	44	1248
3095	3114	525338	GGCGATTGGTGGAGGCAGGA	31	65	1249
3098	3117	525339	TCTGGCGATTGGTGGAGGCA	15	76	1250
3101	3120	525340	CTGTCTGGCGATTGGTGGAG	35	73	1251
3104	3123	525341	TTCCTGTCTGGCGATTGGTG	32	72	1252
3107	3126	525342	GCCTTCCTGTCTGGCGATTG	28	64	1253
3110	3129	525343	GCTGCCTTCCTGTCTGGCGA	25	69	1254

3113	3132	525344	TAGGCTGCCTTCCTGTCTGG	32	79	1255
3116	3135	525345	GGGTAGGCTGCCTTCCTGTC	35	80	1256
3134	3153	525346	TCAAAGGTGGAGACAGCGGG	4	57	1257
3137	3156	525347	TTCTCAAAGGTGGAGACAGC	32	72	1258
3140	3159	525348	TGTTTCTCAAAGGTGGAGAC	32	66	1259
3143	3162	525349	GAGTGTTTCTCAAAGGTGGA	34	63	1260
3146	3165	525350	GATGAGTGTTTCTCAAAGGT	35	68	1261
3149	3168	525351	GAGGATGAGTGTTTCTCAAA	36	84	1262
3152	3171	525352	CCTGAGGATGAGTGTTTCTC	44	77	1263
3155	3174	525353	TGGCCTGAGGATGAGTGTTT	32	72	1264
3158	3177	525354	GCATGGCCTGAGGATGAGTG	27	73	1265
3162	3181	525355	CACTGCATGGCCTGAGGATG	40	69	1266

Table 25

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1286 (RTS3370 and RTS3372)

Viral Start Site	Viral Stop Site	ISIS No	Sequence	RTS3370 % inhibition	RTS3372 % inhibition	SEQ ID NO
85	104	525356	TTCCACTGCATGGCCTGAGG	53	78	1267
88	107	525357	GAATTCCACTGCATGGCCTG	44	68	1268
91	110	525358	GTGGAATTCCACTGCATGGC	42	80	1269
94	113	525359	GTTGTGGAATTCCACTGCAT	45	77	1270
97	116	525360	AAGGTTGTGGAATTCCACTG	65	67	1271
100	119	525361	TGAAAGGTTGTGGAATTCCA	56	61	1272

Example 12: Dose-dependent inhibition of viral HBV RNA in HepG2.2.15 cells by MOE gapmers

Certain gapmers from the study described in Examples 11 and 12 were tested at various doses in human HepG2.2.15 cells. Cells were plated at a density of 28,000 cells per well and transfected using LipofectAMINE 2000® reagent with 5.56 nM, 16.67 nM, 50.0 nM, and 150.0 nM concentrations of antisense oligonucleotide, as specified in Table 26. After a treatment period of approximately 16 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe set RTS3370 was used to measure mRNA levels. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented in Table 26. As illustrated in Table 26, HBV mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 26

Dose-dependent antisense inhibition of HBV RNA in HepG2.2.15 cells using RTS3370

ISIS No	5.5556 nM	16.6667 nM	50.0 nM	150.0 nM	IC_{50} (nM)
146785	0	0	14	66	120.8
146786	40	64	78	88	8.5
505314	23	35	58	84	28.8
505339	28	42	62	84	23.2
505347	9	21	45	75	53.5
514469	11	22	69	79	35.4
524493	13	39	56	81	32.8
524540	15	38	54	80	34.0
524617	14	32	78	83	27.1
524619	33	42	60	84	21.3
524634	20	45	63	80	26.3
524641	39	49	62	86	14.9
524698	34	34	49	64	47.4
524699	25	31	44	63	66.1
524706	29	20	36	58	128.8
524709	32	26	48	56	89.1
524718	46	41	61	79	15.8
524731	49	53	68	83	8.1
524734	42	31	35	64	87.2
524767	19	38	62	84	27.8
524768	35	38	62	75	23.5
524769	16	26	61	75	38.1
524806	0	0	0	35	>150.0
524807	3	22	39	74	60.2
524907	22	35	63	80	29.1
524908	25	45	67	78	22.9
524976	7	3	0	16	>150.0
524978	6	0	0	27	>150.0
524979	3	0	11	34	>150.0
524980	18	51	48	59	51.5
524981	16	27	49	61	65.8
524982	21	19	29	54	>150.0
524983	23	40	50	60	53.2
524984	19	25	45	74	50.0
524985	13	19	40	56	107.2
524986	29	48	46	64	39.3
524987	17	0	43	61	102.8
524988	22	39	52	63	47.6

524991	0	7	19	20	>150.0
524997	17	0	1	9	>150.0
524998	1	5	8	34	>150.0
525095	5	0	0	18	>150.0
525100	14	5	14	26	>150.0
525101	0	0	15	19	>150.0
525102	0	0	18	23	>150.0
525103	0	0	3	15	>150.0
525179	18	7	9	18	>150.0
525245	0	0	8	8	>150.0
525247	12	15	16	23	>150.0
525289	1	1	15	30	>150.0
525314	17	0	18	25	>150.0
525324	0	6	13	16	>150.0
525351	28	13	22	30	>150.0

Some of the ISIS-oligonucleotides were also tested using primer probe set RTS3372. The results are presented in Table 27.

Table 27

Dose-dependent antisense inhibition of HBV RNA in HepG2.2.15 cells using RTS3372

ISIS No	5.5556 nM	16.6667 nM	50.0 nM	150.0 nM	IC_{50} (nM)
146785	0	0	0	51	>150.0
146786	41	68	81	91	7.9
505347	0	13	44	75	59.7
524103	0	0	1	9	>150.0
524245	0	0	6	10	>150.0
524767	18	46	60	85	25.8
524768	34	41	66	79	20.5
524769	12	38	60	77	34.5
524806	0	0	0	0	>150.0
524807	0	9	34	70	78.6
524907	20	41	62	84	26.4
524908	27	45	66	82	21.3
524976	0	0	0	16	>150.0
524978	3	0	0	22	>150.0
524979	0	0	0	33	>150.0
524980	28	51	52	67	30.1
524981	7	29	51	66	55.8
524982	22	29	37	63	83.5
524983	20	51	43	62	50.9

524984	20	30	38	75	51.7
524985	30	33	40	60	83.6
524986	25	51	51	66	33.8
524987	19	0	24	65	157.6
524988	12	41	45	62	59.2
524991	0	0	4	8	>150.0
524997	19	0	0	15	>150.0
524998	0	0	1	42	>150.0
525095	0	0	0	17	>150.0
525100	10	0	4	19	>150.0
525101	10	0	21	25	>150.0
525102	0	0	10	15	>150.0
525247	11	12	15	28	>150.0
525289	0	9	11	33	>150.0
525314	1	0	18	24	>150.0
525324	9	8	15	10	>150.0

Example 13: Dose-dependent inhibition of viral HBV RNA in HepG2.2.15 cells by MOE gapmers

Certain gapmers from the studies described above were tested at various doses in human HepG2.2.15 cells. Cells were plated at a density of 30,000 cells per well and transfected using LipofectAMINE 2000® reagent with 7.8125 nM, 15.625 nM, 31.25 nM, 62.5 nM, 125.0 nM and 250.0 nM concentrations of antisense oligonucleotide, as specified in Table 28. After a treatment period of approximately 16 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe set RTS3370 was used to measure mRNA levels. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented in Table 28. As illustrated in Table 28, HBV mRNA levels were reduced in a dose-dependent manner in several antisense oligonucleotide treated cells.

Table 28

Dose-dependent antisense inhibition of HBV RNA in HepG2.2.15 cells using RTS3370

ISIS No	7.8125 nM	15.625 nM	31.25 nM	62.5 nM	125.0 nM	250.0 nM	IC_{50} (nM)
146786	0	0	14	49	33	50	161.2
510100	0	17	30	28	44	53	177.8
510106	0	4	0	0	29	0	>250.0
509934	0	0	0	7	16	0	>250.0

510116	0	0	8	21	27	25	>250.0
505347	31	3	30	63	80	81	48.7

Example 14: Antisense inhibition of HBV viral mRNA in HepG2 cells by MOE gapmers

Antisense oligonucleotides were designed targeting a HBV viral nucleic acid and were tested for their effects on HBV mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables. ISIS 146786, 509934, ISIS 509959, and ISIS 510100, from the studies described above, were also included. Cultured HepG2 cells at a density of 28,000 cells per well were transfected using LipofectAMINE2000® with 70 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe set RTS3370 (forward sequence CTTGGTCATGGGCCATCAG, designated herein as SEQ ID NO: 2; reverse sequence CGGCTAGGAGTTCCGCAGTA, designated herein as SEQ ID NO: 3; probe sequence TGCGTGGAACCTTTTCGGCTCC, designated herein as SEQ ID NO: 4) was used to measure mRNA levels. Levels were also measured using primer probe set RTS3371 (forward sequence CCAAACCTTCGGACGGAAA, designated herein as SEQ ID NO: 311; reverse sequence TGAGGCCCACTCCCATAGG, designated herein as SEQ ID NO: 312; probe sequence CCCATCATCCTGGGCTTTCGGAAAAT, designated herein as SEQ ID NO: 313). HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells. In some of the assays shown in Tables 32, 35, 42, 45, and 46, the potency of ISIS 146786 was measured in two wells in a single plate. In those cases, the values of inhibition levels in both wells have been presented.

The newly designed chimeric antisense oligonucleotides in Tables below were designed as either 2-9-5 MOE gapmers, 2-9-6 MOE gapmers, 2-10-8 MOE gapmers, 3-9-4 MOE gapmers, 3-9-5 MOE gapmers, 3-10-3 MOE gapmers, 3-10-4 MOE gapmers, 3-10-7 MOE gapmers, 4-9-3 MOE gapmers, 4-9-4 MOE gapmers, 4-10-6 MOE gapmers, 5-9-2 MOE gapmers, 5-9-3 MOE gapmers, 5-10-5 MOE gapmers, 6-9-2 MOE gapmers, 6-10-4 MOE gapmers, 7-10-3 MOE gapmers, or 8-10-2 MOE gapmers. The 2-9-5 MOE gapmers are 16 nucleosides in length, wherein the central gap segment comprises of nine 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising two and five nucleosides respectively. The 2-9-6 MOE gapmers are 17 nucleosides in length, wherein the central gap segment comprises of nine 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising two and six nucleosides respectively. The 2-10-8 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising two and eight nucleosides respectively. The 3-9-4 MOE gapmers are 16 nucleosides in length, wherein the central gap segment comprises of nine 2'-

deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising three and four nucleosides respectively. The 3-9-5 MOE gapmers are 17 nucleosides in length, wherein the central gap segment comprises of nine 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising three and five nucleosides respectively. The 3-10-3 MOE gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising three nucleosides each. The 3-10-4 MOE gapmers are 17 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising three and four nucleosides respectively. The 3-10-7 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising three and seven nucleosides respectively. The 4-9-3 MOE gapmers are 16 nucleosides in length, wherein the central gap segment comprises of nine 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising four and three nucleosides respectively. The 4-9-4 MOE gapmers are 17 nucleosides in length, wherein the central gap segment comprises of nine 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising four nucleosides each. The 4-10-6 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising four and six nucleosides respectively. The 5-9-2 MOE gapmers are 16 nucleosides in length, wherein the central gap segment comprises of nine 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five and two nucleosides respectively. The 5-9-3 MOE gapmers are 17 nucleosides in length, wherein the central gap segment comprises of nine 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five and three nucleosides respectively. The 5-10-5 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleosides each. The 6-9-2 MOE gapmers are 17 nucleosides in length, wherein the central gap segment comprises of nine 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising six and two nucleosides respectively. The 6-10-4 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising six and four nucleosides respectively. The 7-10-3 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising seven and three nucleosides respectively. The 8-10-2 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising eight and two nucleosides respectively. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has an MOE sugar modification. The 'Motif' column indicates the motifs with the number of nucleosides in the wings and the gap segment of each

of the oligonucleotides. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each oligonucleotide are 5-methylcytosines.

"Viral Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the viral gene sequence. "Viral Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted viral gene sequence. The 'Motif' column indicates the gap and wing structure of each gapmer. Each gapmer listed in the Tables is targeted to the viral genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. U95551.1). The potency of the newly designed oligonucleotides was compared with ISIS 146786, 509934, ISIS 509959, and ISIS 510100, the information of which have been placed at the top of each table.

Table 29

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3370

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	50	224
411	427	GGCATAGCAGCAGGATG	510100	3-10-4	62	17
58	74	CTGGAGCCACCAGCAGG	552276	5-9-3	42	1288
59	75	ACTGGAGCCACCAGCAG	552277	5-9-3	46	1289
60	76	AACTGGAGCCACCAGCA	552278	5-9-3	31	1290
61	77	GAACTGGAGCCACCAGC	552279	5-9-3	41	1291
253	269	GAAGTCCACCACGAGTC	552280	5-9-3	5	9
254	270	AGAAGTCCACCACGAGT	552281	5-9-3	11	10
255	271	GAGAAGTCCACCACGAG	552282	5-9-3	20	11
256	272	AGAGAAGTCCACCACGA	552283	5-9-3	28	12
411	427	GGCATAGCAGCAGGATG	552230	4-9-4	57	17
411	427	GGCATAGCAGCAGGATG	552284	5-9-3	0	17
412	428	AGGCATAGCAGCAGGAT	552231	4-9-4	29	18
412	428	AGGCATAGCAGCAGGAT	552285	5-9-3	61	18
413	429	GAGGCATAGCAGCAGGA	552232	4-9-4	35	19
413	429	GAGGCATAGCAGCAGGA	552286	5-9-3	47	19
414	430	TGAGGCATAGCAGCAGG	552233	4-9-4	38	21
414	430	TGAGGCATAGCAGCAGG	552287	5-9-3	45	21
415	431	ATGAGGCATAGCAGCAG	552234	4-9-4	0	23
415	431	ATGAGGCATAGCAGCAG	552288	5-9-3	50	23
416	432	GATGAGGCATAGCAGCA	552235	4-9-4	0	25
416	432	GATGAGGCATAGCAGCA	552289	5-9-3	46	25
417	433	AGATGAGGCATAGCAGC	552236	4-9-4	45	27
417	433	AGATGAGGCATAGCAGC	552290	5-9-3	41	27
418	434	AAGATGAGGCATAGCAG	552237	4-9-4	44	29

418	434	AAGATGAGGCATAGCAG	552291	5-9-3	26	29
670	686	ACTAGTAAACTGAGCCA	552239	4-9-4	62	1292
670	686	ACTAGTAAACTGAGCCA	552293	5-9-3	67	1292
671	687	CACTAGTAAACTGAGCC	552240	4-9-4	61	1293
671	687	CACTAGTAAACTGAGCC	552294	5-9-3	71	1293
672	688	GCACTAGTAAACTGAGC	552241	4-9-4	55	1294
672	688	GCACTAGTAAACTGAGC	552295	5-9-3	58	1294
687	703	ACCACTGAACAAATGGC	552242	4-9-4	60	40
687	703	ACCACTGAACAAATGGC	552296	5-9-3	59	40
688	704	AACCACTGAACAAATGG	552243	4-9-4	57	41
688	704	AACCACTGAACAAATGG	552297	5-9-3	55	41
689	705	GAACCACTGAACAAATG	552244	4-9-4	33	42
689	705	GAACCACTGAACAAATG	552298	5-9-3	48	42
690	706	CGAACCACTGAACAAAT	552245	4-9-4	48	43
690	706	CGAACCACTGAACAAAT	552299	5-9-3	34	43
1261	1277	CGCAGTATGGATCGGCA	552246	4-9-4	81	1295
1261	1277	CGCAGTATGGATCGGCA	552300	5-9-3	56	1295
1262	1278	CCGCAGTATGGATCGGC	552247	4-9-4	87	1296
1262	1278	CCGCAGTATGGATCGGC	552301	5-9-3	86	1296
1263	1279	TCCGCAGTATGGATCGG	552248	4-9-4	72	1297
1263	1279	TCCGCAGTATGGATCGG	552302	5-9-3	77	1297
1264	1280	TTCCGCAGTATGGATCG	552249	4-9-4	56	1298
1264	1280	TTCCGCAGTATGGATCG	552303	5-9-3	65	1298
1265	1281	GTTCCGCAGTATGGATC	552250	4-9-4	52	1299
1265	1281	GTTCCGCAGTATGGATC	552304	5-9-3	57	1299
1266	1282	AGTTCCGCAGTATGGAT	552251	4-9-4	43	1300
1266	1282	AGTTCCGCAGTATGGAT	552305	5-9-3	56	1300
1267	1283	GAGTTCCGCAGTATGGA	552252	4-9-4	62	1301
1267	1283	GAGTTCCGCAGTATGGA	552306	5-9-3	75	1301
1268	1284	GGAGTTCCGCAGTATGG	552253	4-9-4	82	1302
1268	1284	GGAGTTCCGCAGTATGG	552307	5-9-3	90	1302
1269	1285	AGGAGTTCCGCAGTATG	552254	4-9-4	74	1303
1577	1593	AAGCGAAGTGCACACGG	552255	4-9-4	78	1304
1578	1594	GAAGCGAAGTGCACACG	552256	4-9-4	65	1305
1579	1595	TGAAGCGAAGTGCACAC	552257	4-9-4	62	1306
1580	1596	GTGAAGCGAAGTGCACA	552258	4-9-4	72	1307
1581	1597	GGTGAAGCGAAGTGCAC	552259	4-9-4	63	1308
1582	1598	AGGTGAAGCGAAGTGCA	552260	4-9-4	58	1309
1583	1599	GAGGTGAAGCGAAGTGC	552261	4-9-4	63	1310
1584	1600	AGAGGTGAAGCGAAGTG	552262	4-9-4	50	1311
1585	1601	CAGAGGTGAAGCGAAGT	552263	4-9-4	60	1312
1586	1602	GCAGAGGTGAAGCGAAG	552264	4-9-4	52	1313
1587	1603	TGCAGAGGTGAAGCGAA	552265	4-9-4	68	1314

1588	1604	GTGCAGAGGTGAAGCGA	552266	4-9-4	62	1315
1589	1605	CGTGCAGAGGTGAAGCG	552267	4-9-4	58	1316
1590	1606	ACGTGCAGAGGTGAAGC	552268	4-9-4	62	1317
1778	1794	TTATGCCTACAGCCTCC	552269	4-9-4	52	47
1779	1795	TTTATGCCTACAGCCTC	552270	4-9-4	54	49
1780	1796	ATTTATGCCTACAGCCT	552271	4-9-4	58	51
1781	1797	AATTTATGCCTACAGCC	552272	4-9-4	40	53
1782	1798	CAATTTATGCCTACAGC	552273	4-9-4	34	54
1783	1799	CCAATTTATGCCTACAG	552274	4-9-4	34	55
1784	1800	ACCAATTTATGCCTACA	552275	4-9-4	39	56

Table 30

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3370

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	49	224
414	429	GAGGCATAGCAGCAGG	509959	3-10-3	43	145
411	427	GGCATAGCAGCAGGATG	510100	3-10-4	54	17
58	73	TGGAGCCACCAGCAGG	552384	2-9-5	29	1318
58	73	TGGAGCCACCAGCAGG	552440	3-9-4	58	1318
59	74	CTGGAGCCACCAGCAG	552385	2-9-5	57	1319
59	74	CTGGAGCCACCAGCAG	552441	3-9-4	42	1319
60	75	ACTGGAGCCACCAGCA	552386	2-9-5	53	1320
60	75	ACTGGAGCCACCAGCA	552442	3-9-4	53	1320
61	76	AACTGGAGCCACCAGC	552387	2-9-5	48	1321
61	76	AACTGGAGCCACCAGC	552443	3-9-4	59	1321
62	77	GAACTGGAGCCACCAG	552388	2-9-5	40	86
62	77	GAACTGGAGCCACCAG	552444	3-9-4	51	86
411	426	GCATAGCAGCAGGATG	552389	2-9-5	39	137
411	426	GCATAGCAGCAGGATG	552445	3-9-4	60	137
412	427	GGCATAGCAGCAGGAT	552390	2-9-5	52	140
412	427	GGCATAGCAGCAGGAT	552446	3-9-4	54	140
413	428	AGGCATAGCAGCAGGA	552391	2-9-5	57	143
413	428	AGGCATAGCAGCAGGA	552447	3-9-4	54	143
414	429	GAGGCATAGCAGCAGG	552392	2-9-5	0	145
414	429	GAGGCATAGCAGCAGG	552448	3-9-4	58	145
415	430	TGAGGCATAGCAGCAG	552393	2-9-5	59	147
415	430	TGAGGCATAGCAGCAG	552449	3-9-4	60	147
416	431	ATGAGGCATAGCAGCA	552394	2-9-5	53	149
416	431	ATGAGGCATAGCAGCA	552450	3-9-4	53	149
417	432	GATGAGGCATAGCAGC	552395	2-9-5	57	151

417	432	GATGAGGCATAGCAGC	552451	3-9-4	39	151
418	433	AGATGAGGCATAGCAG	552396	2-9-5	62	153
418	433	AGATGAGGCATAGCAG	552452	3-9-4	57	153
457	473	ACGGGCAACATACCTTG	552238	4-9-4	38	33
457	473	ACGGGCAACATACCTTG	552292	5-9-3	48	33
457	473	ACGGGCAACATACCTTG	552346	6-9-2	0	33
457	472	CGGGCAACATACCTTG	552397	2-9-5	63	167
457	472	CGGGCAACATACCTTG	552453	3-9-4	56	167
458	473	ACGGGCAACATACCTT	552398	2-9-5	61	168
458	473	ACGGGCAACATACCTT	552454	3-9-4	48	168
670	685	CTAGTAAACTGAGCCA	552399	2-9-5	52	181
671	686	ACTAGTAAACTGAGCC	552400	2-9-5	57	1322
672	687	CACTAGTAAACTGAGC	552401	2-9-5	52	1323
673	688	GCACTAGTAAACTGAG	552402	2-9-5	54	1324
687	702	CCACTGAACAAATGGC	552403	2-9-5	74	188
688	703	ACCACTGAACAAATGG	552404	2-9-5	43	190
689	704	AACCACTGAACAAATG	552405	2-9-5	15	191
690	705	GAACCACTGAACAAAT	552406	2-9-5	37	192
691	706	CGAACCACTGAACAAA	552407	2-9-5	37	194
1261	1276	GCAGTATGGATCGGCA	552408	2-9-5	76	211
1262	1277	CGCAGTATGGATCGGC	552409	2-9-5	76	1325
1263	1278	CCGCAGTATGGATCGG	552410	2-9-5	63	1326
1264	1279	TCCGCAGTATGGATCG	552411	2-9-5	70	1327
1265	1280	TTCCGCAGTATGGATC	552412	2-9-5	62	1328
1266	1281	GTTCCGCAGTATGGAT	552413	2-9-5	56	1329
1267	1282	AGTTCCGCAGTATGGA	552414	2-9-5	63	1330
1268	1283	GAGTTCCGCAGTATGG	552415	2-9-5	52	1331
1269	1284	GGAGTTCCGCAGTATG	552416	2-9-5	67	1332
1270	1285	AGGAGTTCCGCAGTAT	552417	2-9-5	50	1333
1577	1592	AGCGAAGTGCACACGG	552418	2-9-5	79	1334
1578	1593	AAGCGAAGTGCACACG	552419	2-9-5	70	1335
1579	1594	GAAGCGAAGTGCACAC	552420	2-9-5	71	1336
1580	1595	TGAAGCGAAGTGCACA	552421	2-9-5	69	1337
1581	1596	GTGAAGCGAAGTGCAC	552422	2-9-5	68	1338
1582	1597	GGTGAAGCGAAGTGCA	552423	2-9-5	65	1339
1583	1598	AGGTGAAGCGAAGTGC	552424	2-9-5	70	1340
1584	1599	GAGGTGAAGCGAAGTG	552425	2-9-5	51	1341
1585	1600	AGAGGTGAAGCGAAGT	552426	2-9-5	40	1342
1586	1601	CAGAGGTGAAGCGAAG	552427	2-9-5	35	1343
1587	1602	GCAGAGGTGAAGCGAA	552428	2-9-5	58	1344
1588	1603	TGCAGAGGTGAAGCGA	552429	2-9-5	46	1345
1589	1604	GTGCAGAGGTGAAGCG	552430	2-9-5	53	1346
1590	1605	CGTGCAGAGGTGAAGC	552431	2-9-5	51	1347

1591	1606	ACGTGCAGAGGTGAAG	552432	2-9-5	57	1348
1778	1793	TATGCCTACAGCCTCC	552433	2-9-5	54	230
1779	1794	TTATGCCTACAGCCTC	552434	2-9-5	44	231
1780	1795	TTTATGCCTACAGCCT	552435	2-9-5	46	232
1781	1796	ATTTATGCCTACAGCC	552436	2-9-5	36	233
1782	1797	AATTTATGCCTACAGC	552437	2-9-5	27	234
1783	1798	CAATTTATGCCTACAG	552438	2-9-5	27	235
1784	1799	CCAATTTATGCCTACA	552439	2-9-5	13	236

Table 31

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3370

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	35	224
414	429	GAGGCATAGCAGCAGG	509959	3-10-3	52	145
58	73	TGGAGCCACCAGCAGG	552496	4-9-3	47	1318
59	74	CTGGAGCCACCAGCAG	552497	4-9-3	57	1319
60	75	ACTGGAGCCACCAGCA	552498	4-9-3	45	1320
61	76	AACTGGAGCCACCAGC	552499	4-9-3	52	1321
62	77	GAACTGGAGCCACCAG	552500	4-9-3	46	86
411	426	GCATAGCAGCAGGATG	552501	4-9-3	44	137
412	427	GGCATAGCAGCAGGAT	552502	4-9-3	57	140
413	428	AGGCATAGCAGCAGGA	552503	4-9-3	52	143
414	429	GAGGCATAGCAGCAGG	552504	4-9-3	45	145
415	430	TGAGGCATAGCAGCAG	552505	4-9-3	56	147
416	431	ATGAGGCATAGCAGCA	552506	4-9-3	54	149
417	432	GATGAGGCATAGCAGC	552507	4-9-3	34	151
418	433	AGATGAGGCATAGCAG	552508	4-9-3	34	153
457	472	CGGGCAACATACCTTG	552509	4-9-3	48	167
458	473	ACGGGCAACATACCTT	552510	4-9-3	50	168
670	685	CTAGTAAACTGAGCCA	552455	3-9-4	66	181
670	685	CTAGTAAACTGAGCCA	552511	4-9-3	66	181
671	686	ACTAGTAAACTGAGCC	552456	3-9-4	64	1322
671	686	ACTAGTAAACTGAGCC	552512	4-9-3	62	1322
672	687	CACTAGTAAACTGAGC	552457	3-9-4	14	1323
672	687	CACTAGTAAACTGAGC	552513	4-9-3	56	1323
673	688	GCACTAGTAAACTGAG	552458	3-9-4	59	1324
673	688	GCACTAGTAAACTGAG	552514	4-9-3	52	1324
687	702	CCACTGAACAAATGGC	552459	3-9-4	69	188
687	702	CCACTGAACAAATGGC	552515	4-9-3	57	188
688	703	ACCACTGAACAAATGG	552460	3-9-4	0	190

688	703	ACCACTGAACAAATGG	552516	4-9-3	54	190
689	704	AACCACTGAACAAATG	552461	3-9-4	20	191
689	704	AACCACTGAACAAATG	552517	4-9-3	52	191
690	705	GAACCACTGAACAAAT	552462	3-9-4	46	192
690	705	GAACCACTGAACAAAT	552518	4-9-3	34	192
691	706	CGAACCACTGAACAAA	552463	3-9-4	48	194
691	706	CGAACCACTGAACAAA	552519	4-9-3	44	194
1261	1276	GCAGTATGGATCGGCA	552464	3-9-4	81	211
1261	1276	GCAGTATGGATCGGCA	552520	4-9-3	69	211
1262	1277	CGCAGTATGGATCGGC	552465	3-9-4	84	1325
1262	1277	CGCAGTATGGATCGGC	552521	4-9-3	80	1325
1263	1278	CCGCAGTATGGATCGG	552466	3-9-4	75	1326
1263	1278	CCGCAGTATGGATCGG	552522	4-9-3	76	1326
1264	1279	TCCGCAGTATGGATCG	552467	3-9-4	65	1327
1264	1279	TCCGCAGTATGGATCG	552523	4-9-3	71	1327
1265	1280	TTCCGCAGTATGGATC	552468	3-9-4	53	1328
1265	1280	TTCCGCAGTATGGATC	552524	4-9-3	43	1328
1266	1281	GTTCCGCAGTATGGAT	552469	3-9-4	51	1329
1266	1281	GTTCCGCAGTATGGAT	552525	4-9-3	57	1329
1267	1282	AGTTCCGCAGTATGGA	552470	3-9-4	46	1330
1267	1282	AGTTCCGCAGTATGGA	552526	4-9-3	60	1330
1268	1283	GAGTTCCGCAGTATGG	552471	3-9-4	54	1331
1268	1283	GAGTTCCGCAGTATGG	552527	4-9-3	72	1331
1269	1284	GGAGTTCCGCAGTATG	552472	3-9-4	78	1332
1269	1284	GGAGTTCCGCAGTATG	552528	4-9-3	78	1332
1270	1285	AGGAGTTCCGCAGTAT	552473	3-9-4	67	1333
1270	1285	AGGAGTTCCGCAGTAT	552529	4-9-3	77	1333
1577	1592	AGCGAAGTGCACACGG	552474	3-9-4	79	1334
1577	1592	AGCGAAGTGCACACGG	552530	4-9-3	78	1334
1578	1593	AAGCGAAGTGCACACG	552475	3-9-4	74	1335
1578	1593	AAGCGAAGTGCACACG	552531	4-9-3	68	1335
1579	1594	GAAGCGAAGTGCACAC	552476	3-9-4	52	1336
1580	1595	TGAAGCGAAGTGCACA	552477	3-9-4	76	1337
1581	1596	GTGAAGCGAAGTGCAC	552478	3-9-4	70	1338
1582	1597	GGTGAAGCGAAGTGCA	552479	3-9-4	67	1339
1583	1598	AGGTGAAGCGAAGTGC	552480	3-9-4	68	1340
1584	1599	GAGGTGAAGCGAAGTG	552481	3-9-4	57	1341
1585	1600	AGAGGTGAAGCGAAGT	552482	3-9-4	51	1342
1586	1601	CAGAGGTGAAGCGAAG	552483	3-9-4	48	1343
1587	1602	GCAGAGGTGAAGCGAA	552484	3-9-4	58	1344
1588	1603	TGCAGAGGTGAAGCGA	552485	3-9-4	51	1345
1589	1604	GTGCAGAGGTGAAGCG	552486	3-9-4	55	1346
1590	1605	CGTGCAGAGGTGAAGC	552487	3-9-4	62	1347

1591	1606	ACGTGCAGAGGTGAAG	552488	3-9-4	51	1348
1778	1793	TATGCCTACAGCCTCC	552489	3-9-4	49	230
1779	1794	TTATGCCTACAGCCTC	552490	3-9-4	51	231
1780	1795	TTTATGCCTACAGCCT	552491	3-9-4	51	232
1781	1796	ATTTATGCCTACAGCC	552492	3-9-4	38	233
1782	1797	AATTTATGCCTACAGC	552493	3-9-4	52	234
1783	1798	CAATTTATGCCTACAG	552494	3-9-4	17	235
1784	1799	CCAATTTATGCCTACA	552495	3-9-4	49	236

Table 32

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3370

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	43 52	224
414	429	GAGGCATAGCAGCAGG	509959	3-10-3	38	145
58	73	TGGAGCCACCAGCAGG	552552	5-9-2	33	1318
59	74	CTGGAGCCACCAGCAG	552553	5-9-2	46	1319
60	75	ACTGGAGCCACCAGCA	552554	5-9-2	54	1320
61	76	AACTGGAGCCACCAGC	552555	5-9-2	50	1321
62	77	GAACTGGAGCCACCAG	552556	5-9-2	46	86
411	426	GCATAGCAGCAGGATG	552557	5-9-2	57	137
412	427	GGCATAGCAGCAGGAT	552558	5-9-2	55	140
413	428	AGGCATAGCAGCAGGA	552559	5-9-2	66	143
414	429	GAGGCATAGCAGCAGG	552560	5-9-2	44	145
415	430	TGAGGCATAGCAGCAG	552561	5-9-2	48	147
416	431	ATGAGGCATAGCAGCA	552562	5-9-2	52	149
417	432	GATGAGGCATAGCAGC	552563	5-9-2	45	151
418	433	AGATGAGGCATAGCAG	552564	5-9-2	41	153
457	472	CGGGCAACATACCTTG	552565	5-9-2	54	167
458	473	ACGGGCAACATACCTT	552566	5-9-2	56	168
670	685	CTAGTAAACTGAGCCA	552567	5-9-2	71	181
671	686	ACTAGTAAACTGAGCC	552568	5-9-2	64	1322
672	687	CACTAGTAAACTGAGC	552569	5-9-2	59	1323
673	688	GCACTAGTAAACTGAG	552570	5-9-2	60	1324
687	702	CCACTGAACAAATGGC	552571	5-9-2	55	188
688	703	ACCACTGAACAAATGG	552572	5-9-2	60	190
689	704	AACCACTGAACAAATG	552573	5-9-2	24	191
690	705	GAACCACTGAACAAAT	552574	5-9-2	34	192
691	706	CGAACCACTGAACAAA	552575	5-9-2	36	194
1261	1276	GCAGTATGGATCGGCA	552576	5-9-2	67	211

1262	1277	CGCAGTATGGATCGGC	552577	5-9-2	64	1325
1263	1278	CCGCAGTATGGATCGG	552578	5-9-2	75	1326
1264	1279	TCCGCAGTATGGATCG	552579	5-9-2	75	1327
1265	1280	TTCCGCAGTATGGATC	552580	5-9-2	59	1328
1266	1281	GTTCCGCAGTATGGAT	552581	5-9-2	54	1329
1267	1282	AGTTCCGCAGTATGGA	552582	5-9-2	61	1330
1268	1283	GAGTTCCGCAGTATGG	552583	5-9-2	69	1331
1269	1284	GGAGTTCCGCAGTATG	552584	5-9-2	74	1332
1270	1285	AGGAGTTCCGCAGTAT	552585	5-9-2	62	1333
1577	1592	AGCGAAGTGCACACGG	552586	5-9-2	79	1334
1578	1593	AAGCGAAGTGCACACG	552587	5-9-2	71	1335
1579	1594	GAAGCGAAGTGCACAC	552532	4-9-3	48	1336
1579	1594	GAAGCGAAGTGCACAC	552588	5-9-2	70	1336
1580	1595	TGAAGCGAAGTGCACA	552533	4-9-3	43	1337
1580	1595	TGAAGCGAAGTGCACA	552589	5-9-2	59	1337
1581	1596	GTGAAGCGAAGTGCAC	552534	4-9-3	62	1338
1581	1596	GTGAAGCGAAGTGCAC	552590	5-9-2	70	1338
1582	1597	GGTGAAGCGAAGTGCA	552535	4-9-3	55	1339
1582	1597	GGTGAAGCGAAGTGCA	552591	5-9-2	51	1339
1583	1598	AGGTGAAGCGAAGTGC	552536	4-9-3	3	1340
1583	1598	AGGTGAAGCGAAGTGC	552592	5-9-2	50	1340
1584	1599	GAGGTGAAGCGAAGTG	552537	4-9-3	14	1341
1584	1599	GAGGTGAAGCGAAGTG	552593	5-9-2	46	1341
1585	1600	AGAGGTGAAGCGAAGT	552538	4-9-3	52	1342
1585	1600	AGAGGTGAAGCGAAGT	552594	5-9-2	55	1342
1586	1601	CAGAGGTGAAGCGAAG	552539	4-9-3	47	1343
1586	1601	CAGAGGTGAAGCGAAG	552595	5-9-2	60	1343
1587	1602	GCAGAGGTGAAGCGAA	552540	4-9-3	60	1344
1587	1602	GCAGAGGTGAAGCGAA	552596	5-9-2	63	1344
1588	1603	TGCAGAGGTGAAGCGA	552541	4-9-3	60	1345
1588	1603	TGCAGAGGTGAAGCGA	552597	5-9-2	61	1345
1589	1604	GTGCAGAGGTGAAGCG	552542	4-9-3	64	1346
1589	1604	GTGCAGAGGTGAAGCG	552598	5-9-2	57	1346
1590	1605	CGTGCAGAGGTGAAGC	552543	4-9-3	46	1347
1590	1605	CGTGCAGAGGTGAAGC	552600	5-9-2	59	1347
1591	1606	ACGTGCAGAGGTGAAG	552544	4-9-3	53	1348
1591	1606	ACGTGCAGAGGTGAAG	552602	5-9-2	6	1348
1778	1793	TATGCCTACAGCCTCC	552545	4-9-3	33	230
1778	1793	TATGCCTACAGCCTCC	552604	5-9-2	47	230
1779	1794	TTATGCCTACAGCCTC	552546	4-9-3	42	231
1779	1794	TTATGCCTACAGCCTC	552606	5-9-2	53	231
1780	1795	TTTATGCCTACAGCCT	552547	4-9-3	51	232
1780	1795	TTTATGCCTACAGCCT	552608	5-9-2	53	232

1781	1796	ATTTATGCCTACAGCC	552548	4-9-3	52	233
1781	1796	ATTTATGCCTACAGCC	552610	5-9-2	47	233
1782	1797	AATTTATGCCTACAGC	552549	4-9-3	38	234
1782	1797	AATTTATGCCTACAGC	552612	5-9-2	39	234
1783	1798	CAATTTATGCCTACAG	552550	4-9-3	19	235
1783	1798	CAATTTATGCCTACAG	552614	5-9-2	24	235
1784	1799	CCAATTTATGCCTACA	552551	4-9-3	24	236
1784	1799	CCAATTTATGCCTACA	552616	5-9-2	15	236

Table 33

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3370

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	51	224
1780	1799	CCAATTTATGCCTACAGCCT	509934	5-10-5	76	50
58	77	GAACTGGAGCCACCAGCAGG	552007	6-10-4	61	83
58	77	GAACTGGAGCCACCAGCAGG	552039	7-10-3	84	83
253	272	AGAGAAGTCCACCACGAGTC	552008	6-10-4	48	103
253	272	AGAGAAGTCCACCACGAGTC	552040	7-10-3	48	103
411	430	TGAGGCATAGCAGCAGGATG	552009	6-10-4	77	136
411	430	TGAGGCATAGCAGCAGGATG	552041	7-10-3	73	136
412	431	ATGAGGCATAGCAGCAGGAT	552010	6-10-4	63	139
412	431	ATGAGGCATAGCAGCAGGAT	552042	7-10-3	66	139
413	432	GATGAGGCATAGCAGCAGGA	552011	6-10-4	52	142
413	432	GATGAGGCATAGCAGCAGGA	552043	7-10-3	54	142
414	433	AGATGAGGCATAGCAGCAGG	552012	6-10-4	73	20
414	433	AGATGAGGCATAGCAGCAGG	552044	7-10-3	86	20
415	434	AAGATGAGGCATAGCAGCAG	552013	6-10-4	73	22
415	434	AAGATGAGGCATAGCAGCAG	552045	7-10-3	65	22
416	435	GAAGATGAGGCATAGCAGCA	552014	6-10-4	76	24
416	435	GAAGATGAGGCATAGCAGCA	552046	7-10-3	93	24
417	436	AGAAGATGAGGCATAGCAGC	552015	6-10-4	70	26
417	436	AGAAGATGAGGCATAGCAGC	552047	7-10-3	77	26
418	437	AAGAAGATGAGGCATAGCAG	552016	6-10-4	61	28
418	437	AAGAAGATGAGGCATAGCAG	552048	7-10-3	66	28
687	706	CGAACCACTGAACAAATGGC	552017	6-10-4	73	39
687	706	CGAACCACTGAACAAATGGC	552049	7-10-3	73	39
1261	1280	TTCCGCAGTATGGATCGGCA	552018	6-10-4	98	719
1261	1280	TTCCGCAGTATGGATCGGCA	552050	7-10-3	98	719
1262	1281	GTTCCGCAGTATGGATCGGC	552019	6-10-4	98	212
1262	1281	GTTCCGCAGTATGGATCGGC	552051	7-10-3	99	212

1263	1282	AGTTCCGCAGTATGGATCGG	551986	4-10-6	92	720
1263	1282	AGTTCCGCAGTATGGATCGG	552020	6-10-4	97	720
1263	1282	AGTTCCGCAGTATGGATCGG	552052	7-10-3	98	720
1264	1283	GAGTTCCGCAGTATGGATCG	551987	4-10-6	95	721
1264	1283	GAGTTCCGCAGTATGGATCG	552021	6-10-4	97	721
1264	1283	GAGTTCCGCAGTATGGATCG	552053	7-10-3	98	721
1265	1284	GGAGTTCCGCAGTATGGATC	551988	4-10-6	50	1349
1265	1284	GGAGTTCCGCAGTATGGATC	552005	5-10-5	99	1349
1265	1284	GGAGTTCCGCAGTATGGATC	552022	6-10-4	99	1349
1265	1284	GGAGTTCCGCAGTATGGATC	552054	7-10-3	99	1349
1266	1285	AGGAGTTCCGCAGTATGGAT	551989	4-10-6	96	722
1266	1285	AGGAGTTCCGCAGTATGGAT	552023	6-10-4	99	722
1266	1285	AGGAGTTCCGCAGTATGGAT	552055	7-10-3	98	722
1577	1596	GTGAAGCGAAGTGCACACGG	551990	4-10-6	86	224
1577	1596	GTGAAGCGAAGTGCACACGG	552024	6-10-4	89	224
1577	1596	GTGAAGCGAAGTGCACACGG	552056	7-10-3	88	224
1578	1597	GGTGAAGCGAAGTGCACACG	551991	4-10-6	0	801
1578	1597	GGTGAAGCGAAGTGCACACG	552025	6-10-4	90	801
1578	1597	GGTGAAGCGAAGTGCACACG	552057	7-10-3	92	801
1579	1598	AGGTGAAGCGAAGTGCACAC	551992	4-10-6	72	802
1579	1598	AGGTGAAGCGAAGTGCACAC	552026	6-10-4	88	802
1579	1598	AGGTGAAGCGAAGTGCACAC	552058	7-10-3	86	802
1580	1599	GAGGTGAAGCGAAGTGCACA	551993	4-10-6	82	225
1580	1599	GAGGTGAAGCGAAGTGCACA	552027	6-10-4	87	225
1580	1599	GAGGTGAAGCGAAGTGCACA	552059	7-10-3	88	225
1581	1600	AGAGGTGAAGCGAAGTGCAC	551994	4-10-6	85	804
1581	1600	AGAGGTGAAGCGAAGTGCAC	552028	6-10-4	83	804
1581	1600	AGAGGTGAAGCGAAGTGCAC	552060	7-10-3	82	804
1582	1601	CAGAGGTGAAGCGAAGTGCA	551995	4-10-6	84	805
1582	1601	CAGAGGTGAAGCGAAGTGCA	552029	6-10-4	88	805
1582	1601	CAGAGGTGAAGCGAAGTGCA	552061	7-10-3	85	805
1583	1602	GCAGAGGTGAAGCGAAGTGC	551996	4-10-6	87	226
1583	1602	GCAGAGGTGAAGCGAAGTGC	552030	6-10-4	88	226
1583	1602	GCAGAGGTGAAGCGAAGTGC	552062	7-10-3	85	226
1584	1603	TGCAGAGGTGAAGCGAAGTG	551997	4-10-6	83	806
1584	1603	TGCAGAGGTGAAGCGAAGTG	552031	6-10-4	82	806
1585	1604	GTGCAGAGGTGAAGCGAAGT	551998	4-10-6	85	807
1585	1604	GTGCAGAGGTGAAGCGAAGT	552032	6-10-4	87	807
1586	1605	CGTGCAGAGGTGAAGCGAAG	551999	4-10-6	82	227
1586	1605	CGTGCAGAGGTGAAGCGAAG	552033	6-10-4	87	227
1587	1606	ACGTGCAGAGGTGAAGCGAA	552000	4-10-6	83	1350
1587	1606	ACGTGCAGAGGTGAAGCGAA	552006	5-10-5	88	1350
1587	1606	ACGTGCAGAGGTGAAGCGAA	552034	6-10-4	89	1350

1778	1797	AATTTATGCCTACAGCCTCC	552001	4-10-6	65	46
1778	1797	AATTTATGCCTACAGCCTCC	552035	6-10-4	60	46
1779	1798	CAATTTATGCCTACAGCCTC	552002	4-10-6	63	48
1779	1798	CAATTTATGCCTACAGCCTC	552036	6-10-4	65	48
1780	1799	CCAATTTATGCCTACAGCCT	552003	4-10-6	65	50
1780	1799	CCAATTTATGCCTACAGCCT	552037	6-10-4	58	50
1781	1800	ACCAATTTATGCCTACAGCC	552004	4-10-6	58	52
1781	1800	ACCAATTTATGCCTACAGCC	552038	6-10-4	70	52

Table 34

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3370

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	64	224
411	427	GGCATAGCAGCAGGATG	510100	3-10-4	62	17
58	74	CTGGAGCCACCAGCAGG	552168	3-9-5	79	1288
58	74	CTGGAGCCACCAGCAGG	552222	4-9-4	79	1288
59	75	ACTGGAGCCACCAGCAG	552169	3-9-5	67	1289
59	75	ACTGGAGCCACCAGCAG	552223	4-9-4	40	1289
60	76	AACTGGAGCCACCAGCA	552170	3-9-5	69	1290
60	76	AACTGGAGCCACCAGCA	552224	4-9-4	64	1290
61	77	GAACTGGAGCCACCAGC	552171	3-9-5	65	1291
61	77	GAACTGGAGCCACCAGC	552225	4-9-4	69	1291
253	269	GAAGTCCACCACGAGTC	552172	3-9-5	33	9
253	269	GAAGTCCACCACGAGTC	552226	4-9-4	48	9
254	270	AGAAGTCCACCACGAGT	552173	3-9-5	41	10
254	270	AGAAGTCCACCACGAGT	552227	4-9-4	32	10
255	271	GAGAAGTCCACCACGAG	552174	3-9-5	31	11
255	271	GAGAAGTCCACCACGAG	552228	4-9-4	42	11
256	272	AGAGAAGTCCACCACGA	552175	3-9-5	59	12
411	427	GGCATAGCAGCAGGATG	552176	3-9-5	68	17
412	428	AGGCATAGCAGCAGGAT	552177	3-9-5	55	18
413	429	GAGGCATAGCAGCAGGA	552178	3-9-5	66	19
414	430	TGAGGCATAGCAGCAGG	552179	3-9-5	70	21
415	431	ATGAGGCATAGCAGCAG	552180	3-9-5	66	23
416	432	GATGAGGCATAGCAGCA	552181	3-9-5	51	25
417	433	AGATGAGGCATAGCAGC	552182	3-9-5	69	27
418	434	AAGATGAGGCATAGCAG	552183	3-9-5	69	29
457	473	ACGGGCAACATACCTTG	552184	3-9-5	43	33
670	686	ACTAGTAAACTGAGCCA	552185	3-9-5	66	1292
671	687	CACTAGTAAACTGAGCC	552186	3-9-5	54	1293

672	688	GCACTAGTAAACTGAGC	552187	3-9-5	74	1294
687	703	ACCACTGAACAAATGGC	552188	3-9-5	78	40
688	704	AACCACTGAACAAATGG	552189	3-9-5	57	41
689	705	GAACCACTGAACAAATG	552190	3-9-5	39	42
690	706	CGAACCACTGAACAAAT	552191	3-9-5	60	43
1261	1277	CGCAGTATGGATCGGCA	552192	3-9-5	85	1295
1262	1278	CCGCAGTATGGATCGGC	552193	3-9-5	86	1296
1263	1279	TCCGCAGTATGGATCGG	552194	3-9-5	68	1297
1264	1280	TTCCGCAGTATGGATCG	552195	3-9-5	73	1298
1265	1281	GTTCCGCAGTATGGATC	552196	3-9-5	60	1299
1266	1282	AGTTCCGCAGTATGGAT	552197	3-9-5	60	1300
1267	1283	GAGTTCCGCAGTATGGA	552198	3-9-5	61	1301
1268	1284	GGAGTTCCGCAGTATGG	552199	3-9-5	89	1302
1269	1285	AGGAGTTCCGCAGTATG	552200	3-9-5	85	1303
1577	1593	AAGCGAAGTGCACACGG	552201	3-9-5	81	1304
1578	1594	GAAGCGAAGTGCACACG	552202	3-9-5	76	1305
1579	1595	TGAAGCGAAGTGCACAC	552203	3-9-5	74	1306
1580	1596	GTGAAGCGAAGTGCACA	552204	3-9-5	71	1307
1581	1597	GGTGAAGCGAAGTGCAC	552151	2-9-6	77	1308
1581	1597	GGTGAAGCGAAGTGCAC	552205	3-9-5	78	1308
1582	1598	AGGTGAAGCGAAGTGCA	552152	2-9-6	72	1309
1582	1598	AGGTGAAGCGAAGTGCA	552206	3-9-5	77	1309
1583	1599	GAGGTGAAGCGAAGTGC	552153	2-9-6	67	1310
1583	1599	GAGGTGAAGCGAAGTGC	552207	3-9-5	81	1310
1584	1600	AGAGGTGAAGCGAAGTG	552154	2-9-6	56	1311
1584	1600	AGAGGTGAAGCGAAGTG	552208	3-9-5	70	1311
1585	1601	CAGAGGTGAAGCGAAGT	552155	2-9-6	61	1312
1585	1601	CAGAGGTGAAGCGAAGT	552209	3-9-5	63	1312
1586	1602	GCAGAGGTGAAGCGAAG	552156	2-9-6	20	1313
1586	1602	GCAGAGGTGAAGCGAAG	552210	3-9-5	75	1313
1587	1603	TGCAGAGGTGAAGCGAA	552157	2-9-6	39	1314
1587	1603	TGCAGAGGTGAAGCGAA	552211	3-9-5	75	1314
1588	1604	GTGCAGAGGTGAAGCGA	552158	2-9-6	70	1315
1588	1604	GTGCAGAGGTGAAGCGA	552212	3-9-5	67	1315
1589	1605	CGTGCAGAGGTGAAGCG	552159	2-9-6	74	1316
1589	1605	CGTGCAGAGGTGAAGCG	552213	3-9-5	70	1316
1590	1606	ACGTGCAGAGGTGAAGC	552160	2-9-6	78	1317
1590	1606	ACGTGCAGAGGTGAAGC	552214	3-9-5	79	1317
1778	1794	TTATGCCTACAGCCTCC	552161	2-9-6	56	47
1778	1794	TTATGCCTACAGCCTCC	552215	3-9-5	61	47
1779	1795	TTTATGCCTACAGCCTC	552162	2-9-6	64	49
1779	1795	TTTATGCCTACAGCCTC	552216	3-9-5	62	49
1780	1796	ATTTATGCCTACAGCCT	552163	2-9-6	71	51

1780	1796	ATTTATGCCTACAGCCT	552217	3-9-5	58	51
1781	1797	AATTTATGCCTACAGCC	552164	2-9-6	52	53
1781	1797	AATTTATGCCTACAGCC	552218	3-9-5	56	53
1782	1798	CAATTTATGCCTACAGC	552165	2-9-6	53	54
1782	1798	CAATTTATGCCTACAGC	552219	3-9-5	33	54
1783	1799	CCAATTTATGCCTACAG	552166	2-9-6	41	55
1783	1799	CCAATTTATGCCTACAG	552220	3-9-5	53	55
1784	1800	ACCAATTTATGCCTACA	552167	2-9-6	54	56
1784	1800	ACCAATTTATGCCTACA	552221	3-9-5	31	56

Table 35

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3370

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	60 85	224
1780	1799	CCAATTTATGCCTACAGCCT	509934	5-10-5	76	50
411	427	GGCATAGCAGCAGGATG	510100	3-10-4	73	17
58	77	GAACTGGAGCCACCAGCAGG	552071	8-10-2	79	83
58	74	CTGGAGCCACCAGCAGG	552114	2-9-6	66	1288
59	75	ACTGGAGCCACCAGCAG	552115	2-9-6	70	1289
60	76	AACTGGAGCCACCAGCA	552116	2-9-6	68	1290
61	77	GAACTGGAGCCACCAGC	552117	2-9-6	70	1291
253	272	AGAGAAGTCCACCACGAGTC	552072	8-10-2	50	103
253	269	GAAGTCCACCACGAGTC	552118	2-9-6	66	9
254	270	AGAAGTCCACCACGAGT	552119	2-9-6	62	10
255	271	GAGAAGTCCACCACGAG	552120	2-9-6	35	11
256	272	AGAGAAGTCCACCACGA	552121	2-9-6	39	12
411	430	TGAGGCATAGCAGCAGGATG	552073	8-10-2	80	136
411	427	GGCATAGCAGCAGGATG	552122	2-9-6	55	17
412	431	ATGAGGCATAGCAGCAGGAT	552074	8-10-2	73	139
412	428	AGGCATAGCAGCAGGAT	552123	2-9-6	75	18
413	432	GATGAGGCATAGCAGCAGGA	552075	8-10-2	78	142
413	429	GAGGCATAGCAGCAGGA	552124	2-9-6	64	19
414	433	AGATGAGGCATAGCAGCAGG	552076	8-10-2	70	20
414	430	TGAGGCATAGCAGCAGG	552125	2-9-6	73	21
415	434	AAGATGAGGCATAGCAGCAG	552077	8-10-2	83	22
415	431	ATGAGGCATAGCAGCAG	552126	2-9-6	64	23
416	435	GAAGATGAGGCATAGCAGCA	552078	8-10-2	80	24
416	432	GATGAGGCATAGCAGCA	552127	2-9-6	72	25
417	436	AGAAGATGAGGCATAGCAGC	552079	8-10-2	86	26

417	433	AGATGAGGCATAGCAGC	552128	2-9-6	76	27
418	437	AAGAAGATGAGGCATAGCAG	552080	8-10-2	83	28
418	434	AAGATGAGGCATAGCAG	552129	2-9-6	72	29
670	686	ACTAGTAAACTGAGCCA	552131	2-9-6	61	1292
671	687	CACTAGTAAACTGAGCC	552132	2-9-6	73	1293
672	688	GCACTAGTAAACTGAGC	552133	2-9-6	75	1294
687	706	CGAACCACTGAACAAATGGC	552081	8-10-2	76	39
687	703	ACCACTGAACAAATGGC	552134	2-9-6	58	40
688	704	AACCACTGAACAAATGG	552135	2-9-6	67	41
689	705	GAACCACTGAACAAATG	552136	2-9-6	65	42
690	706	CGAACCACTGAACAAAT	552137	2-9-6	55	43
1261	1280	TTCCGCAGTATGGATCGGCA	552082	8-10-2	98	719
1261	1277	CGCAGTATGGATCGGCA	552138	2-9-6	82	1295
1262	1281	GTTCCGCAGTATGGATCGGC	552083	8-10-2	99	212
1262	1278	CCGCAGTATGGATCGGC	552139	2-9-6	86	1296
1263	1282	AGTTCCGCAGTATGGATCGG	552084	8-10-2	99	720
1263	1279	TCCGCAGTATGGATCGG	552140	2-9-6	74	1297
1264	1283	GAGTTCCGCAGTATGGATCG	552085	8-10-2	100	721
1264	1280	TTCCGCAGTATGGATCG	552141	2-9-6	67	1298
1265	1284	GGAGTTCCGCAGTATGGATC	552086	8-10-2	100	1349
1265	1281	GTTCCGCAGTATGGATC	552142	2-9-6	45	1299
1266	1285	AGGAGTTCCGCAGTATGGAT	552087	8-10-2	100	722
1266	1282	AGTTCCGCAGTATGGAT	552143	2-9-6	68	1300
1267	1283	GAGTTCCGCAGTATGGA	552144	2-9-6	78	1301
1268	1284	GGAGTTCCGCAGTATGG	552145	2-9-6	88	1302
1269	1285	AGGAGTTCCGCAGTATG	552146	2-9-6	81	1303
1577	1596	GTGAAGCGAAGTGCACACGG	552088	8-10-2	95	224
1577	1593	AAGCGAAGTGCACACGG	552147	2-9-6	88	1304
1578	1597	GGTGAAGCGAAGTGCACACG	552089	8-10-2	93	801
1578	1594	GAAGCGAAGTGCACACG	552148	2-9-6	79	1305
1579	1598	AGGTGAAGCGAAGTGCACAC	552090	8-10-2	87	802
1579	1595	TGAAGCGAAGTGCACAC	552149	2-9-6	81	1306
1580	1599	GAGGTGAAGCGAAGTGCACA	552091	8-10-2	88	225
1581	1600	AGAGGTGAAGCGAAGTGCAC	552092	8-10-2	90	804
1582	1601	CAGAGGTGAAGCGAAGTGCA	552093	8-10-2	91	805
1583	1602	GCAGAGGTGAAGCGAAGTGC	552094	8-10-2	88	226
1584	1603	TGCAGAGGTGAAGCGAAGTG	552063	7-10-3	81	806
1584	1603	TGCAGAGGTGAAGCGAAGTG	552095	8-10-2	89	806
1585	1604	GTGCAGAGGTGAAGCGAAGT	552064	7-10-3	85	807
1585	1604	GTGCAGAGGTGAAGCGAAGT	552096	8-10-2	92	807
1586	1605	CGTGCAGAGGTGAAGCGAAG	552065	7-10-3	86	227
1586	1605	CGTGCAGAGGTGAAGCGAAG	552097	8-10-2	93	227
1587	1606	ACGTGCAGAGGTGAAGCGAA	552066	7-10-3	33	1350

1587	1606	ACGTGCAGAGGTGAAGCGAA	552098	8-10-2	88	1350
1778	1797	AATTTATGCCTACAGCCTCC	552067	7-10-3	50	46
1778	1797	AATTTATGCCTACAGCCTCC	552099	8-10-2	70	46
1779	1798	CAATTTATGCCTACAGCCTC	552068	7-10-3	73	48
1779	1798	CAATTTATGCCTACAGCCTC	552100	8-10-2	70	48
1780	1799	CCAATTTATGCCTACAGCCT	552069	7-10-3	73	50
1780	1799	CCAATTTATGCCTACAGCCT	552101	8-10-2	76	50
1781	1800	ACCAATTTATGCCTACAGCC	552070	7-10-3	71	52
1781	1800	ACCAATTTATGCCTACAGCC	552102	8-10-2	64	52

Table 36

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3370

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	84	224
411	427	GGCATAGCAGCAGGATG	510100	3-10-4	76	17
58	74	CTGGAGCCACCAGCAGG	552330	6-9-2	54	1288
59	75	ACTGGAGCCACCAGCAG	552331	6-9-2	66	1289
60	76	AACTGGAGCCACCAGCA	552332	6-9-2	70	1290
61	77	GAACTGGAGCCACCAGC	552333	6-9-2	55	1291
253	269	GAAGTCCACCACGAGTC	552334	6-9-2	42	9
254	270	AGAAGTCCACCACGAGT	552335	6-9-2	39	10
255	271	GAGAAGTCCACCACGAG	552336	6-9-2	27	11
256	272	AGAGAAGTCCACCACGA	552337	6-9-2	74	12
411	427	GGCATAGCAGCAGGATG	552338	6-9-2	68	17
412	428	AGGCATAGCAGCAGGAT	552339	6-9-2	71	18
413	429	GAGGCATAGCAGCAGGA	552340	6-9-2	61	19
414	430	TGAGGCATAGCAGCAGG	552341	6-9-2	58	21
415	431	ATGAGGCATAGCAGCAG	552342	6-9-2	55	23
416	432	GATGAGGCATAGCAGCA	552343	6-9-2	63	25
417	433	AGATGAGGCATAGCAGC	552344	6-9-2	51	27
418	434	AAGATGAGGCATAGCAG	552345	6-9-2	65	29
457	473	ACGGGCAACATACCTTG	552346	6-9-2	0	33
670	686	ACTAGTAAACTGAGCCA	552347	6-9-2	84	1292
671	687	CACTAGTAAACTGAGCC	552348	6-9-2	87	1293
672	688	GCACTAGTAAACTGAGC	552349	6-9-2	74	1294
687	703	ACCACTGAACAAATGGC	552350	6-9-2	59	40
688	704	AACCACTGAACAAATGG	552351	6-9-2	60	41
689	705	GAACCACTGAACAAATG	552352	6-9-2	53	42
690	706	CGAACCACTGAACAAAT	552353	6-9-2	0	43
1261	1277	CGCAGTATGGATCGGCA	552354	6-9-2	83	1295

1262	1278	CCGCAGTATGGATCGGC	552355	6-9-2	90	1296
1263	1279	TCCGCAGTATGGATCGG	552356	6-9-2	0	1297
1264	1280	TTCCGCAGTATGGATCG	552357	6-9-2	45	1298
1265	1281	GTTCCGCAGTATGGATC	552358	6-9-2	74	1299
1266	1282	AGTTCCGCAGTATGGAT	552359	6-9-2	72	1300
1267	1283	GAGTTCCGCAGTATGGA	552360	6-9-2	87	1301
1268	1284	GGAGTTCCGCAGTATGG	552361	6-9-2	96	1302
1269	1285	AGGAGTTCCGCAGTATG	552308	5-9-3	81	1303
1269	1285	AGGAGTTCCGCAGTATG	552362	6-9-2	92	1303
1577	1593	AAGCGAAGTGCACACGG	552309	5-9-3	77	1304
1577	1593	AAGCGAAGTGCACACGG	552363	6-9-2	92	1304
1578	1594	GAAGCGAAGTGCACACG	552310	5-9-3	80	1305
1578	1594	GAAGCGAAGTGCACACG	552364	6-9-2	87	1305
1579	1595	TGAAGCGAAGTGCACAC	552311	5-9-3	13	1306
1579	1595	TGAAGCGAAGTGCACAC	552365	6-9-2	84	1306
1580	1596	GTGAAGCGAAGTGCACA	552150	2-9-6	73	1307
1580	1596	GTGAAGCGAAGTGCACA	552312	5-9-3	77	1307
1580	1596	GTGAAGCGAAGTGCACA	552366	6-9-2	87	1307
1581	1597	GGTGAAGCGAAGTGCAC	552313	5-9-3	64	1308
1581	1597	GGTGAAGCGAAGTGCAC	552367	6-9-2	85	1308
1582	1598	AGGTGAAGCGAAGTGCA	552314	5-9-3	73	1309
1582	1598	AGGTGAAGCGAAGTGCA	552368	6-9-2	77	1309
1583	1599	GAGGTGAAGCGAAGTGC	552315	5-9-3	75	1310
1583	1599	GAGGTGAAGCGAAGTGC	552369	6-9-2	75	1310
1584	1600	AGAGGTGAAGCGAAGTG	552316	5-9-3	64	1311
1584	1600	AGAGGTGAAGCGAAGTG	552370	6-9-2	63	1311
1585	1601	CAGAGGTGAAGCGAAGT	552317	5-9-3	99	1312
1585	1601	CAGAGGTGAAGCGAAGT	552371	6-9-2	81	1312
1586	1602	GCAGAGGTGAAGCGAAG	552318	5-9-3	76	1313
1586	1602	GCAGAGGTGAAGCGAAG	552372	6-9-2	65	1313
1587	1603	TGCAGAGGTGAAGCGAA	552319	5-9-3	55	1314
1587	1603	TGCAGAGGTGAAGCGAA	552373	6-9-2	74	1314
1588	1604	GTGCAGAGGTGAAGCGA	552320	5-9-3	68	1315
1588	1604	GTGCAGAGGTGAAGCGA	552374	6-9-2	78	1315
1589	1605	CGTGCAGAGGTGAAGCG	552321	5-9-3	74	1316
1589	1605	CGTGCAGAGGTGAAGCG	552375	6-9-2	81	1316
1590	1606	ACGTGCAGAGGTGAAGC	552322	5-9-3	73	1317
1590	1606	ACGTGCAGAGGTGAAGC	552376	6-9-2	78	1317
1778	1794	TTATGCCTACAGCCTCC	552323	5-9-3	75	47
1778	1794	TTATGCCTACAGCCTCC	552377	6-9-2	70	47
1779	1795	TTTATGCCTACAGCCTC	552324	5-9-3	0	49
1779	1795	TTTATGCCTACAGCCTC	552378	6-9-2	72	49
1780	1796	ATTTATGCCTACAGCCT	552325	5-9-3	70	51

1780	1796	ATTTATGCCTACAGCCT	552379	6-9-2	74	51
1781	1797	AATTTATGCCTACAGCC	552326	5-9-3	63	53
1781	1797	AATTTATGCCTACAGCC	552380	6-9-2	53	53
1782	1798	CAATTTATGCCTACAGC	552327	5-9-3	30	54
1782	1798	CAATTTATGCCTACAGC	552381	6-9-2	26	54
1783	1799	CCAATTTATGCCTACAG	552328	5-9-3	25	55
1783	1799	CCAATTTATGCCTACAG	552382	6-9-2	13	55
1784	1800	ACCAATTTATGCCTACA	552329	5-9-3	33	56
1784	1800	ACCAATTTATGCCTACA	552383	6-9-2	5	56

Table 37

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3370

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1780	1799	CCAATTTATGCCTACAGCCT	509934	5-10-5	30	50
58	77	GAACTGGAGCCACCAGCAGG	551909	2-10-8	62	83
58	77	GAACTGGAGCCACCAGCAGG	551941	3-10-7	74	83
58	77	GAACTGGAGCCACCAGCAGG	551973	4-10-6	64	83
253	272	AGAGAAGTCCACCACGAGTC	551910	2-10-8	52	103
253	272	AGAGAAGTCCACCACGAGTC	551942	3-10-7	54	103
253	272	AGAGAAGTCCACCACGAGTC	551974	4-10-6	51	103
411	430	TGAGGCATAGCAGCAGGATG	551911	2-10-8	58	136
411	430	TGAGGCATAGCAGCAGGATG	551943	3-10-7	64	136
411	430	TGAGGCATAGCAGCAGGATG	551975	4-10-6	57	136
412	431	ATGAGGCATAGCAGCAGGAT	551912	2-10-8	59	139
412	431	ATGAGGCATAGCAGCAGGAT	551944	3-10-7	66	139
412	431	ATGAGGCATAGCAGCAGGAT	551976	4-10-6	57	139
413	432	GATGAGGCATAGCAGCAGGA	551913	2-10-8	58	142
413	432	GATGAGGCATAGCAGCAGGA	551945	3-10-7	56	142
413	432	GATGAGGCATAGCAGCAGGA	551977	4-10-6	56	142
414	433	AGATGAGGCATAGCAGCAGG	551914	2-10-8	0	20
414	433	AGATGAGGCATAGCAGCAGG	551946	3-10-7	48	20
414	433	AGATGAGGCATAGCAGCAGG	551978	4-10-6	53	20
415	434	AAGATGAGGCATAGCAGCAG	551915	2-10-8	44	22
415	434	AAGATGAGGCATAGCAGCAG	551947	3-10-7	53	22
415	434	AAGATGAGGCATAGCAGCAG	551979	4-10-6	64	22
416	435	GAAGATGAGGCATAGCAGCA	551916	2-10-8	57	24
416	435	GAAGATGAGGCATAGCAGCA	551948	3-10-7	68	24
416	435	GAAGATGAGGCATAGCAGCA	551980	4-10-6	56	24
417	436	AGAAGATGAGGCATAGCAGC	551917	2-10-8	58	26
417	436	AGAAGATGAGGCATAGCAGC	551949	3-10-7	64	26

417	436	AGAAGATGAGGCATAGCAGC	551981	4-10-6	63	26
418	437	AAGAAGATGAGGCATAGCAG	551918	2-10-8	59	28
418	437	AAGAAGATGAGGCATAGCAG	551950	3-10-7	71	28
418	437	AAGAAGATGAGGCATAGCAG	551982	4-10-6	63	28
687	706	CGAACCACTGAACAAATGGC	551919	2-10-8	76	39
687	706	CGAACCACTGAACAAATGGC	551951	3-10-7	71	39
687	706	CGAACCACTGAACAAATGGC	551983	4-10-6	73	39
1261	1280	TTCCGCAGTATGGATCGGCA	551920	2-10-8	68	719
1261	1280	TTCCGCAGTATGGATCGGCA	551952	3-10-7	76	719
1261	1280	TTCCGCAGTATGGATCGGCA	551984	4-10-6	81	719
1262	1281	GTTCCGCAGTATGGATCGGC	551921	2-10-8	83	212
1262	1281	GTTCCGCAGTATGGATCGGC	551953	3-10-7	82	212
1262	1281	GTTCCGCAGTATGGATCGGC	551985	4-10-6	76	212
1263	1282	AGTTCCGCAGTATGGATCGG	551922	2-10-8	73	720
1263	1282	AGTTCCGCAGTATGGATCGG	551954	3-10-7	68	720
1264	1283	GAGTTCCGCAGTATGGATCG	551923	2-10-8	59	721
1264	1283	GAGTTCCGCAGTATGGATCG	551955	3-10-7	71	721
1265	1284	GGAGTTCCGCAGTATGGATC	551924	2-10-8	80	1349
1265	1284	GGAGTTCCGCAGTATGGATC	551956	3-10-7	80	1349
1266	1285	AGGAGTTCCGCAGTATGGAT	551925	2-10-8	82	722
1266	1285	AGGAGTTCCGCAGTATGGAT	551957	3-10-7	88	722
1577	1596	GTGAAGCGAAGTGCACACGG	551926	2-10-8	71	224
1577	1596	GTGAAGCGAAGTGCACACGG	551958	3-10-7	74	224
1578	1597	GGTGAAGCGAAGTGCACACG	551927	2-10-8	68	801
1578	1597	GGTGAAGCGAAGTGCACACG	551959	3-10-7	69	801
1579	1598	AGGTGAAGCGAAGTGCACAC	551928	2-10-8	69	802
1579	1598	AGGTGAAGCGAAGTGCACAC	551960	3-10-7	62	802
1580	1599	GAGGTGAAGCGAAGTGCACA	551929	2-10-8	54	225
1580	1599	GAGGTGAAGCGAAGTGCACA	551961	3-10-7	20	225
1581	1600	AGAGGTGAAGCGAAGTGCAC	551930	2-10-8	53	804
1581	1600	AGAGGTGAAGCGAAGTGCAC	551962	3-10-7	60	804
1582	1601	CAGAGGTGAAGCGAAGTGCA	551931	2-10-8	47	805
1582	1601	CAGAGGTGAAGCGAAGTGCA	551963	3-10-7	63	805
1583	1602	GCAGAGGTGAAGCGAAGTGC	551932	2-10-8	68	226
1583	1602	GCAGAGGTGAAGCGAAGTGC	551964	3-10-7	56	226
1584	1603	TGCAGAGGTGAAGCGAAGTG	551933	2-10-8	72	806
1584	1603	TGCAGAGGTGAAGCGAAGTG	551965	3-10-7	67	806
1585	1604	GTGCAGAGGTGAAGCGAAGT	551934	2-10-8	64	807
1585	1604	GTGCAGAGGTGAAGCGAAGT	551966	3-10-7	73	807
1586	1605	CGTGCAGAGGTGAAGCGAAG	551935	2-10-8	68	227
1586	1605	CGTGCAGAGGTGAAGCGAAG	551967	3-10-7	60	227
1587	1606	ACGTGCAGAGGTGAAGCGAA	551936	2-10-8	67	1350
1587	1606	ACGTGCAGAGGTGAAGCGAA	551968	3-10-7	63	1350

1778	1797	AATTTATGCCTACAGCCTCC	551937	2-10-8	47	46
1778	1797	AATTTATGCCTACAGCCTCC	551969	3-10-7	36	46
1779	1798	CAATTTATGCCTACAGCCTC	551938	2-10-8	41	48
1779	1798	CAATTTATGCCTACAGCCTC	551970	3-10-7	43	48
1780	1799	CCAATTTATGCCTACAGCCT	551939	2-10-8	53	50
1780	1799	CCAATTTATGCCTACAGCCT	551971	3-10-7	55	50
1781	1800	ACCAATTTATGCCTACAGCC	551940	2-10-8	50	52
1781	1800	ACCAATTTATGCCTACAGCC	551972	3-10-7	58	52

Table 38

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3371

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1780	1799	CCAATTTATGCCTACAGCCT	509934	5-10-5	21	50
58	77	GAACTGGAGCCACCAGCAGG	551909	2-10-8	52	83
58	77	GAACTGGAGCCACCAGCAGG	551941	3-10-7	62	83
58	77	GAACTGGAGCCACCAGCAGG	551973	4-10-6	58	83
253	272	AGAGAAGTCCACCACGAGTC	551910	2-10-8	48	103
253	272	AGAGAAGTCCACCACGAGTC	551942	3-10-7	36	103
253	272	AGAGAAGTCCACCACGAGTC	551974	4-10-6	45	103
411	430	TGAGGCATAGCAGCAGGATG	551911	2-10-8	61	136
411	430	TGAGGCATAGCAGCAGGATG	551943	3-10-7	56	136
411	430	TGAGGCATAGCAGCAGGATG	551975	4-10-6	60	136
412	431	ATGAGGCATAGCAGCAGGAT	551912	2-10-8	53	139
412	431	ATGAGGCATAGCAGCAGGAT	551944	3-10-7	48	139
412	431	ATGAGGCATAGCAGCAGGAT	551976	4-10-6	48	139
413	432	GATGAGGCATAGCAGCAGGA	551913	2-10-8	53	142
413	432	GATGAGGCATAGCAGCAGGA	551945	3-10-7	54	142
413	432	GATGAGGCATAGCAGCAGGA	551977	4-10-6	48	142
414	433	AGATGAGGCATAGCAGCAGG	551914	2-10-8	0	20
414	433	AGATGAGGCATAGCAGCAGG	551946	3-10-7	56	20
414	433	AGATGAGGCATAGCAGCAGG	551978	4-10-6	36	20
415	434	AAGATGAGGCATAGCAGCAG	551915	2-10-8	47	22
415	434	AAGATGAGGCATAGCAGCAG	551947	3-10-7	45	22
415	434	AAGATGAGGCATAGCAGCAG	551979	4-10-6	54	22
416	435	GAAGATGAGGCATAGCAGCA	551916	2-10-8	44	24
416	435	GAAGATGAGGCATAGCAGCA	551948	3-10-7	59	24
416	435	GAAGATGAGGCATAGCAGCA	551980	4-10-6	49	24
417	436	AGAAGATGAGGCATAGCAGC	551917	2-10-8	48	26
417	436	AGAAGATGAGGCATAGCAGC	551949	3-10-7	60	26
417	436	AGAAGATGAGGCATAGCAGC	551981	4-10-6	57	26

418	437	AAGAAGATGAGGCATAGCAG	551918	2-10-8	53	28
418	437	AAGAAGATGAGGCATAGCAG	551950	3-10-7	57	28
418	437	AAGAAGATGAGGCATAGCAG	551982	4-10-6	57	28
687	706	CGAACCACTGAACAAATGGC	551919	2-10-8	65	39
687	706	CGAACCACTGAACAAATGGC	551951	3-10-7	57	39
687	706	CGAACCACTGAACAAATGGC	551983	4-10-6	53	39
1261	1280	TTCCGCAGTATGGATCGGCA	551920	2-10-8	57	719
1261	1280	TTCCGCAGTATGGATCGGCA	551952	3-10-7	67	719
1261	1280	TTCCGCAGTATGGATCGGCA	551984	4-10-6	62	719
1262	1281	GTTCCGCAGTATGGATCGGC	551921	2-10-8	60	212
1262	1281	GTTCCGCAGTATGGATCGGC	551953	3-10-7	57	212
1262	1281	GTTCCGCAGTATGGATCGGC	551985	4-10-6	58	212
1263	1282	AGTTCCGCAGTATGGATCGG	551922	2-10-8	63	720
1263	1282	AGTTCCGCAGTATGGATCGG	551954	3-10-7	61	720
1264	1283	GAGTTCCGCAGTATGGATCG	551923	2-10-8	50	721
1264	1283	GAGTTCCGCAGTATGGATCG	551955	3-10-7	44	721
1265	1284	GGAGTTCCGCAGTATGGATC	551924	2-10-8	52	1349
1265	1284	GGAGTTCCGCAGTATGGATC	551956	3-10-7	46	1349
1266	1285	AGGAGTTCCGCAGTATGGAT	551925	2-10-8	54	722
1266	1285	AGGAGTTCCGCAGTATGGAT	551957	3-10-7	51	722
1577	1596	GTGAAGCGAAGTGCACACGG	551926	2-10-8	70	224
1577	1596	GTGAAGCGAAGTGCACACGG	551958	3-10-7	72	224
1578	1597	GGTGAAGCGAAGTGCACACG	551927	2-10-8	60	801
1578	1597	GGTGAAGCGAAGTGCACACG	551959	3-10-7	61	801
1579	1598	AGGTGAAGCGAAGTGCACAC	551928	2-10-8	57	802
1579	1598	AGGTGAAGCGAAGTGCACAC	551960	3-10-7	58	802
1580	1599	GAGGTGAAGCGAAGTGCACA	551929	2-10-8	49	225
1580	1599	GAGGTGAAGCGAAGTGCACA	551961	3-10-7	26	225
1581	1600	AGAGGTGAAGCGAAGTGCAC	551930	2-10-8	54	804
1581	1600	AGAGGTGAAGCGAAGTGCAC	551962	3-10-7	57	804
1582	1601	CAGAGGTGAAGCGAAGTGCA	551931	2-10-8	46	805
1582	1601	CAGAGGTGAAGCGAAGTGCA	551963	3-10-7	56	805
1583	1602	GCAGAGGTGAAGCGAAGTGC	551932	2-10-8	57	226
1583	1602	GCAGAGGTGAAGCGAAGTGC	551964	3-10-7	53	226
1584	1603	TGCAGAGGTGAAGCGAAGTG	551933	2-10-8	65	806
1584	1603	TGCAGAGGTGAAGCGAAGTG	551965	3-10-7	54	806
1585	1604	GTGCAGAGGTGAAGCGAAGT	551934	2-10-8	58	807
1585	1604	GTGCAGAGGTGAAGCGAAGT	551966	3-10-7	69	807
1586	1605	CGTGCAGAGGTGAAGCGAAG	551935	2-10-8	63	227
1586	1605	CGTGCAGAGGTGAAGCGAAG	551967	3-10-7	53	227
1587	1606	ACGTGCAGAGGTGAAGCGAA	551936	2-10-8	67	1350
1587	1606	ACGTGCAGAGGTGAAGCGAA	551968	3-10-7	60	1350
1778	1797	AATTTATGCCTACAGCCTCC	551937	2-10-8	51	46

1778	1797	AATTTATGCCTACAGCCTCC	551969	3-10-7	42	46
1779	1798	CAATTTATGCCTACAGCCTC	551938	2-10-8	40	48
1779	1798	CAATTTATGCCTACAGCCTC	551970	3-10-7	38	48
1780	1799	CCAATTTATGCCTACAGCCT	551939	2-10-8	32	50
1780	1799	CCAATTTATGCCTACAGCCT	551971	3-10-7	46	50
1781	1800	ACCAATTTATGCCTACAGCC	551940	2-10-8	39	52
1781	1800	ACCAATTTATGCCTACAGCC	551972	3-10-7	51	52

Table 39

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3371

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	40	224
411	427	GGCATAGCAGCAGGATG	510100	3-10-4	60	17
58	74	CTGGAGCCACCAGCAGG	552276	5-9-3	44	1288
59	75	ACTGGAGCCACCAGCAG	552277	5-9-3	39	1289
60	76	AACTGGAGCCACCAGCA	552278	5-9-3	37	1290
61	77	GAACTGGAGCCACCAGC	552279	5-9-3	50	1291
253	269	GAAGTCCACCACGAGTC	552280	5-9-3	2	9
254	270	AGAAGTCCACCACGAGT	552281	5-9-3	0	10
255	271	GAGAAGTCCACCACGAG	552282	5-9-3	13	11
256	272	AGAGAAGTCCACCACGA	552229	4-9-4	17	12
256	272	AGAGAAGTCCACCACGA	552283	5-9-3	27	12
411	427	GGCATAGCAGCAGGATG	552230	4-9-4	53	17
411	427	GGCATAGCAGCAGGATG	552284	5-9-3	0	17
412	428	AGGCATAGCAGCAGGAT	552231	4-9-4	31	18
412	428	AGGCATAGCAGCAGGAT	552285	5-9-3	56	18
413	429	GAGGCATAGCAGCAGGA	552232	4-9-4	35	19
413	429	GAGGCATAGCAGCAGGA	552286	5-9-3	43	19
414	430	TGAGGCATAGCAGCAGG	552233	4-9-4	40	21
414	430	TGAGGCATAGCAGCAGG	552287	5-9-3	44	21
415	431	ATGAGGCATAGCAGCAG	552234	4-9-4	0	23
415	431	ATGAGGCATAGCAGCAG	552288	5-9-3	44	23
416	432	GATGAGGCATAGCAGCA	552235	4-9-4	13	25
416	432	GATGAGGCATAGCAGCA	552289	5-9-3	21	25
417	433	AGATGAGGCATAGCAGC	552236	4-9-4	40	27
417	433	AGATGAGGCATAGCAGC	552290	5-9-3	34	27
418	434	AAGATGAGGCATAGCAG	552237	4-9-4	37	29
418	434	AAGATGAGGCATAGCAG	552291	5-9-3	34	29
670	686	ACTAGTAAACTGAGCCA	552239	4-9-4	58	1292
670	686	ACTAGTAAACTGAGCCA	552293	5-9-3	61	1292

671	687	CACTAGTAAACTGAGCC	552240	4-9-4	54	1293
671	687	CACTAGTAAACTGAGCC	552294	5-9-3	62	1293
672	688	GCACTAGTAAACTGAGC	552241	4-9-4	47	1294
672	688	GCACTAGTAAACTGAGC	552295	5-9-3	63	1294
687	703	ACCACTGAACAAATGGC	552242	4-9-4	61	40
687	703	ACCACTGAACAAATGGC	552296	5-9-3	61	40
688	704	AACCACTGAACAAATGG	552243	4-9-4	55	41
688	704	AACCACTGAACAAATGG	552297	5-9-3	52	41
689	705	GAACCACTGAACAAATG	552244	4-9-4	45	42
689	705	GAACCACTGAACAAATG	552298	5-9-3	27	42
690	706	CGAACCACTGAACAAAT	552245	4-9-4	41	43
690	706	CGAACCACTGAACAAAT	552299	5-9-3	32	43
1261	1277	CGCAGTATGGATCGGCA	552246	4-9-4	67	1295
1261	1277	CGCAGTATGGATCGGCA	552300	5-9-3	57	1295
1262	1278	CCGCAGTATGGATCGGC	552247	4-9-4	74	1296
1262	1278	CCGCAGTATGGATCGGC	552301	5-9-3	76	1296
1263	1279	TCCGCAGTATGGATCGG	552248	4-9-4	65	1297
1263	1279	TCCGCAGTATGGATCGG	552302	5-9-3	68	1297
1264	1280	TTCCGCAGTATGGATCG	552249	4-9-4	38	1298
1264	1280	TTCCGCAGTATGGATCG	552303	5-9-3	59	1298
1265	1281	GTTCCGCAGTATGGATC	552250	4-9-4	43	1299
1265	1281	GTTCCGCAGTATGGATC	552304	5-9-3	30	1299
1266	1282	AGTTCCGCAGTATGGAT	552251	4-9-4	52	1300
1266	1282	AGTTCCGCAGTATGGAT	552305	5-9-3	49	1300
1267	1283	GAGTTCCGCAGTATGGA	552252	4-9-4	51	1301
1267	1283	GAGTTCCGCAGTATGGA	552306	5-9-3	56	1301
1268	1284	GGAGTTCCGCAGTATGG	552253	4-9-4	47	1302
1268	1284	GGAGTTCCGCAGTATGG	552307	5-9-3	49	1302
1269	1285	AGGAGTTCCGCAGTATG	552254	4-9-4	50	1303
1577	1593	AAGCGAAGTGCACACGG	552255	4-9-4	64	1304
1578	1594	GAAGCGAAGTGCACACG	552256	4-9-4	57	1305
1579	1595	TGAAGCGAAGTGCACAC	552257	4-9-4	51	1306
1580	1596	GTGAAGCGAAGTGCACA	552258	4-9-4	62	1307
1581	1597	GGTGAAGCGAAGTGCAC	552259	4-9-4	59	1308
1582	1598	AGGTGAAGCGAAGTGCA	552260	4-9-4	56	1309
1583	1599	GAGGTGAAGCGAAGTGC	552261	4-9-4	54	1310
1584	1600	AGAGGTGAAGCGAAGTG	552262	4-9-4	47	1311
1585	1601	CAGAGGTGAAGCGAAGT	552263	4-9-4	45	1312
1586	1602	GCAGAGGTGAAGCGAAG	552264	4-9-4	52	1313
1587	1603	TGCAGAGGTGAAGCGAA	552265	4-9-4	58	1314
1588	1604	GTGCAGAGGTGAAGCGA	552266	4-9-4	54	1315
1589	1605	CGTGCAGAGGTGAAGCG	552267	4-9-4	43	1316
1590	1606	ACGTGCAGAGGTGAAGC	552268	4-9-4	57	1317

1778	1794	TTATGCCTACAGCCTCC	552269	4-9-4	34	47
1779	1795	TTTATGCCTACAGCCTC	552270	4-9-4	37	49
1780	1796	ATTTATGCCTACAGCCT	552271	4-9-4	42	51
1781	1797	AATTTATGCCTACAGCC	552272	4-9-4	36	53
1782	1798	CAATTTATGCCTACAGC	552273	4-9-4	25	54
1783	1799	CCAATTTATGCCTACAG	552274	4-9-4	11	55
1784	1800	ACCAATTTATGCCTACA	552275	4-9-4	38	56

Table 40

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3371

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	38	1354
414	429	GAGGCATAGCAGCAGG	509959	3-10-3	49	145
411	427	GGCATAGCAGCAGGATG	510100	3-10-4	55	17
58	73	TGGAGCCACCAGCAGG	552384	2-9-5	41	1318
58	73	TGGAGCCACCAGCAGG	552440	3-9-4	57	1318
59	74	CTGGAGCCACCAGCAG	552385	2-9-5	53	1319
59	74	CTGGAGCCACCAGCAG	552441	3-9-4	38	1319
60	75	ACTGGAGCCACCAGCA	552386	2-9-5	42	1320
60	75	ACTGGAGCCACCAGCA	552442	3-9-4	72	1320
61	76	AACTGGAGCCACCAGC	552387	2-9-5	43	1321
61	76	AACTGGAGCCACCAGC	552443	3-9-4	56	1321
62	77	GAACTGGAGCCACCAG	552388	2-9-5	18	86
62	77	GAACTGGAGCCACCAG	552444	3-9-4	39	86
411	426	GCATAGCAGCAGGATG	552389	2-9-5	24	137
411	426	GCATAGCAGCAGGATG	552445	3-9-4	53	137
412	427	GGCATAGCAGCAGGAT	552390	2-9-5	40	140
412	427	GGCATAGCAGCAGGAT	552446	3-9-4	57	140
413	428	AGGCATAGCAGCAGGA	552391	2-9-5	51	143
413	428	AGGCATAGCAGCAGGA	552447	3-9-4	53	143
414	429	GAGGCATAGCAGCAGG	552392	2-9-5	0	145
414	429	GAGGCATAGCAGCAGG	552448	3-9-4	57	145
415	430	TGAGGCATAGCAGCAG	552393	2-9-5	52	147
415	430	TGAGGCATAGCAGCAG	552449	3-9-4	49	147
416	431	ATGAGGCATAGCAGCA	552394	2-9-5	32	149
416	431	ATGAGGCATAGCAGCA	552450	3-9-4	44	149
417	432	GATGAGGCATAGCAGC	552395	2-9-5	33	151
417	432	GATGAGGCATAGCAGC	552451	3-9-4	38	151
418	433	AGATGAGGCATAGCAG	552396	2-9-5	46	153
418	433	AGATGAGGCATAGCAG	552452	3-9-4	30	153

457	473	ACGGGCAACATACCTTG	552130	2-9-6	46	33
457	473	ACGGGCAACATACCTTG	552184	3-9-5	34	33
457	473	ACGGGCAACATACCTTG	552238	4-9-4	41	33
457	473	ACGGGCAACATACCTTG	552292	5-9-3	45	33
457	473	ACGGGCAACATACCTTG	552346	6-9-2	0	33
457	472	CGGGCAACATACCTTG	552397	2-9-5	37	167
457	472	CGGGCAACATACCTTG	552453	3-9-4	45	167
458	473	ACGGGCAACATACCTT	552398	2-9-5	42	168
458	473	ACGGGCAACATACCTT	552454	3-9-4	39	168
670	685	CTAGTAAACTGAGCCA	552399	2-9-5	34	181
671	686	ACTAGTAAACTGAGCC	552400	2-9-5	47	1322
672	687	CACTAGTAAACTGAGC	552401	2-9-5	53	1323
673	688	GCACTAGTAAACTGAG	552402	2-9-5	47	1324
687	702	CCACTGAACAAATGGC	552403	2-9-5	70	188
688	703	ACCACTGAACAAATGG	552404	2-9-5	44	190
689	704	AACCACTGAACAAATG	552405	2-9-5	0	191
690	705	GAACCACTGAACAAAT	552406	2-9-5	25	192
691	706	CGAACCACTGAACAAA	552407	2-9-5	23	194
1261	1276	GCAGTATGGATCGGCA	552408	2-9-5	73	211
1262	1277	CGCAGTATGGATCGGC	552409	2-9-5	71	1325
1263	1278	CCGCAGTATGGATCGG	552410	2-9-5	52	1326
1264	1279	TCCGCAGTATGGATCG	552411	2-9-5	62	1327
1265	1280	TTCCGCAGTATGGATC	552412	2-9-5	50	1328
1266	1281	GTTCCGCAGTATGGAT	552413	2-9-5	55	1329
1267	1282	AGTTCCGCAGTATGGA	552414	2-9-5	64	1330
1268	1283	GAGTTCCGCAGTATGG	552415	2-9-5	45	1331
1269	1284	GGAGTTCCGCAGTATG	552416	2-9-5	45	1332
1270	1285	AGGAGTTCCGCAGTAT	552417	2-9-5	37	1333
1577	1592	AGCGAAGTGCACACGG	552418	2-9-5	73	1334
1578	1593	AAGCGAAGTGCACACG	552419	2-9-5	68	1335
1579	1594	GAAGCGAAGTGCACAC	552420	2-9-5	64	1336
1580	1595	TGAAGCGAAGTGCACA	552421	2-9-5	54	1337
1581	1596	GTGAAGCGAAGTGCAC	552422	2-9-5	60	1338
1582	1597	GGTGAAGCGAAGTGCA	552423	2-9-5	62	1339
1583	1598	AGGTGAAGCGAAGTGC	552424	2-9-5	60	1340
1584	1599	GAGGTGAAGCGAAGTG	552425	2-9-5	46	1341
1585	1600	AGAGGTGAAGCGAAGT	552426	2-9-5	48	1342
1586	1601	CAGAGGTGAAGCGAAG	552427	2-9-5	36	1343
1587	1602	GCAGAGGTGAAGCGAA	552428	2-9-5	57	1344
1588	1603	TGCAGAGGTGAAGCGA	552429	2-9-5	36	1345
1589	1604	GTGCAGAGGTGAAGCG	552430	2-9-5	42	1346
1590	1605	CGTGCAGAGGTGAAGC	552431	2-9-5	60	1347
1591	1606	ACGTGCAGAGGTGAAG	552432	2-9-5	44	1348

1778	1793	TATGCCTACAGCCTCC	552433	2-9-5	55	230
1779	1794	TTATGCCTACAGCCTC	552434	2-9-5	46	231
1780	1795	TTTATGCCTACAGCCT	552435	2-9-5	47	232
1781	1796	ATTTATGCCTACAGCC	552436	2-9-5	25	233
1782	1797	AATTTATGCCTACAGC	552437	2-9-5	19	234
1783	1798	CAATTTATGCCTACAG	552438	2-9-5	25	235
1784	1799	CCAATTTATGCCTACA	552439	2-9-5	22	236

Table 41

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3371

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
414	429	GAGGCATAGCAGCAGG	509959	3-10-3	49	145
58	73	TGGAGCCACCAGCAGG	552496	4-9-3	35	1318
59	74	CTGGAGCCACCAGCAG	552497	4-9-3	60	1319
60	75	ACTGGAGCCACCAGCA	552498	4-9-3	20	1320
61	76	AACTGGAGCCACCAGC	552499	4-9-3	45	1321
62	77	GAACTGGAGCCACCAG	552500	4-9-3	53	86
411	426	GCATAGCAGCAGGATG	552501	4-9-3	56	137
412	427	GGCATAGCAGCAGGAT	552502	4-9-3	50	140
413	428	AGGCATAGCAGCAGGA	552503	4-9-3	36	143
414	429	GAGGCATAGCAGCAGG	552504	4-9-3	50	145
415	430	TGAGGCATAGCAGCAG	552505	4-9-3	53	147
416	431	ATGAGGCATAGCAGCA	552506	4-9-3	49	149
417	432	GATGAGGCATAGCAGC	552507	4-9-3	35	151
418	433	AGATGAGGCATAGCAG	552508	4-9-3	62	153
457	472	CGGGCAACATACCTTG	552509	4-9-3	65	167
458	473	ACGGGCAACATACCTT	552510	4-9-3	54	168
670	685	CTAGTAAACTGAGCCA	552455	3-9-4	60	181
670	685	CTAGTAAACTGAGCCA	552511	4-9-3	65	181
671	686	ACTAGTAAACTGAGCC	552456	3-9-4	69	1322
671	686	ACTAGTAAACTGAGCC	552512	4-9-3	63	1322
672	687	CACTAGTAAACTGAGC	552457	3-9-4	4	1323
672	687	CACTAGTAAACTGAGC	552513	4-9-3	50	1323
673	688	GCACTAGTAAACTGAG	552458	3-9-4	59	1324
673	688	GCACTAGTAAACTGAG	552514	4-9-3	53	1324
687	702	CCACTGAACAAATGGC	552459	3-9-4	69	188
687	702	CCACTGAACAAATGGC	552515	4-9-3	68	188
688	703	ACCACTGAACAAATGG	552460	3-9-4	3	190
688	703	ACCACTGAACAAATGG	552516	4-9-3	65	190
689	704	AACCACTGAACAAATG	552461	3-9-4	37	191

689	704	AACCACTGAACAAATG	552517	4-9-3	54	191
690	705	GAACCACTGAACAAAT	552462	3-9-4	42	192
690	705	GAACCACTGAACAAAT	552518	4-9-3	23	192
691	706	CGAACCACTGAACAAA	552463	3-9-4	28	194
691	706	CGAACCACTGAACAAA	552519	4-9-3	32	194
1261	1276	GCAGTATGGATCGGCA	552464	3-9-4	72	211
1261	1276	GCAGTATGGATCGGCA	552520	4-9-3	61	211
1262	1277	CGCAGTATGGATCGGC	552465	3-9-4	68	1325
1262	1277	CGCAGTATGGATCGGC	552521	4-9-3	68	1325
1263	1278	CCGCAGTATGGATCGG	552466	3-9-4	76	1326
1263	1278	CCGCAGTATGGATCGG	552522	4-9-3	71	1326
1264	1279	TCCGCAGTATGGATCG	552467	3-9-4	72	1327
1264	1279	TCCGCAGTATGGATCG	552523	4-9-3	73	1327
1265	1280	TTCCGCAGTATGGATC	552468	3-9-4	50	1328
1265	1280	TTCCGCAGTATGGATC	552524	4-9-3	49	1328
1266	1281	GTTCCGCAGTATGGAT	552469	3-9-4	65	1329
1266	1281	GTTCCGCAGTATGGAT	552525	4-9-3	45	1329
1267	1282	AGTTCCGCAGTATGGA	552470	3-9-4	58	1330
1267	1282	AGTTCCGCAGTATGGA	552526	4-9-3	39	1330
1268	1283	GAGTTCCGCAGTATGG	552471	3-9-4	30	1331
1268	1283	GAGTTCCGCAGTATGG	552527	4-9-3	39	1331
1269	1284	GGAGTTCCGCAGTATG	552472	3-9-4	43	1332
1269	1284	GGAGTTCCGCAGTATG	552528	4-9-3	43	1332
1270	1285	AGGAGTTCCGCAGTAT	552473	3-9-4	25	1333
1270	1285	AGGAGTTCCGCAGTAT	552529	4-9-3	50	1333
1577	1592	AGCGAAGTGCACACGG	552474	3-9-4	70	1334
1577	1592	AGCGAAGTGCACACGG	552530	4-9-3	73	1334
1578	1593	AAGCGAAGTGCACACG	552475	3-9-4	64	1335
1578	1593	AAGCGAAGTGCACACG	552531	4-9-3	62	1335
1579	1594	GAAGCGAAGTGCACAC	552476	3-9-4	50	1336
1580	1595	TGAAGCGAAGTGCACA	552477	3-9-4	66	1337
1581	1596	GTGAAGCGAAGTGCAC	552478	3-9-4	68	1338
1582	1597	GGTGAAGCGAAGTGCA	552479	3-9-4	60	1339
1583	1598	AGGTGAAGCGAAGTGC	552480	3-9-4	58	1340
1584	1599	GAGGTGAAGCGAAGTG	552481	3-9-4	54	1341
1585	1600	AGAGGTGAAGCGAAGT	552482	3-9-4	44	1342
1586	1601	CAGAGGTGAAGCGAAG	552483	3-9-4	17	1343
1587	1602	GCAGAGGTGAAGCGAA	552484	3-9-4	64	1344
1588	1603	TGCAGAGGTGAAGCGA	552485	3-9-4	56	1345
1589	1604	GTGCAGAGGTGAAGCG	552486	3-9-4	26	1346
1590	1605	CGTGCAGAGGTGAAGC	552487	3-9-4	42	1347
1591	1606	ACGTGCAGAGGTGAAG	552488	3-9-4	35	1348
1778	1793	TATGCCTACAGCCTCC	552489	3-9-4	46	230

1779	1794	TTATGCCTACAGCCTC	552490	3-9-4	41	231
1780	1795	TTTATGCCTACAGCCT	552491	3-9-4	38	232
1781	1796	ATTTATGCCTACAGCC	552492	3-9-4	47	233
1782	1797	AATTTATGCCTACAGC	552493	3-9-4	49	234
1783	1798	CAATTTATGCCTACAG	552494	3-9-4	22	235
1784	1799	CCAATTTATGCCTACA	552495	3-9-4	0	236

Table 42

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3371

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	56 55	224
414	429	GAGGCATAGCAGCAGG	509959	3-10-3	54	145
58	73	TGGAGCCACCAGCAGG	552552	5-9-2	32	1355
59	74	CTGGAGCCACCAGCAG	552553	5-9-2	53	1319
60	75	ACTGGAGCCACCAGCA	552554	5-9-2	48	1320
61	76	AACTGGAGCCACCAGC	552555	5-9-2	39	1321
62	77	GAACTGGAGCCACCAG	552556	5-9-2	39	86
411	426	GCATAGCAGCAGGATG	552557	5-9-2	54	137
412	427	GGCATAGCAGCAGGAT	552558	5-9-2	41	140
413	428	AGGCATAGCAGCAGGA	552559	5-9-2	56	143
414	429	GAGGCATAGCAGCAGG	552560	5-9-2	39	145
415	430	TGAGGCATAGCAGCAG	552561	5-9-2	51	147
416	431	ATGAGGCATAGCAGCA	552562	5-9-2	56	149
417	432	GATGAGGCATAGCAGC	552563	5-9-2	31	151
418	433	AGATGAGGCATAGCAG	552564	5-9-2	31	153
457	472	CGGGCAACATACCTTG	552565	5-9-2	53	167
458	473	ACGGGCAACATACCTT	552566	5-9-2	46	168
670	685	CTAGTAAACTGAGCCA	552567	5-9-2	63	181
671	686	ACTAGTAAACTGAGCC	552568	5-9-2	66	1322
672	687	CACTAGTAAACTGAGC	552569	5-9-2	60	1323
673	688	GCACTAGTAAACTGAG	552570	5-9-2	60	1324
687	702	CCACTGAACAAATGGC	552571	5-9-2	44	188
688	703	ACCACTGAACAAATGG	552572	5-9-2	52	190
689	704	AACCACTGAACAAATG	552573	5-9-2	20	191
690	705	GAACCACTGAACAAAT	552574	5-9-2	36	192
691	706	CGAACCACTGAACAAA	552575	5-9-2	19	194
1261	1276	GCAGTATGGATCGGCA	552576	5-9-2	61	211
1262	1277	CGCAGTATGGATCGGC	552577	5-9-2	57	1325
1263	1278	CCGCAGTATGGATCGG	552578	5-9-2	71	1326

1264	1279	TCCGCAGTATGGATCG	552579	5-9-2	59	1327
1265	1280	TTCCGCAGTATGGATC	552580	5-9-2	58	1328
1266	1281	GTTCCGCAGTATGGAT	552581	5-9-2	51	1329
1267	1282	AGTTCCGCAGTATGGA	552582	5-9-2	40	1330
1268	1283	GAGTTCCGCAGTATGG	552583	5-9-2	35	1331
1269	1284	GGAGTTCCGCAGTATG	552584	5-9-2	50	1332
1270	1285	AGGAGTTCCGCAGTAT	552585	5-9-2	48	1333
1577	1592	AGCGAAGTGCACACGG	552586	5-9-2	74	1334
1578	1593	AAGCGAAGTGCACACG	552587	5-9-2	68	1335
1579	1594	GAAGCGAAGTGCACAC	552532	4-9-3	59	1336
1579	1594	GAAGCGAAGTGCACAC	552588	5-9-2	67	1336
1580	1595	TGAAGCGAAGTGCACA	552533	4-9-3	52	1337
1580	1595	TGAAGCGAAGTGCACA	552589	5-9-2	47	1337
1581	1596	GTGAAGCGAAGTGCAC	552534	4-9-3	71	1338
1581	1596	GTGAAGCGAAGTGCAC	552590	5-9-2	58	1338
1582	1597	GGTGAAGCGAAGTGCA	552535	4-9-3	59	1339
1582	1597	GGTGAAGCGAAGTGCA	552591	5-9-2	46	1339
1583	1598	AGGTGAAGCGAAGTGC	552536	4-9-3	19	1340
1583	1598	AGGTGAAGCGAAGTGC	552592	5-9-2	44	1340
1584	1599	GAGGTGAAGCGAAGTG	552537	4-9-3	26	1341
1584	1599	GAGGTGAAGCGAAGTG	552593	5-9-2	39	1341
1585	1600	AGAGGTGAAGCGAAGT	552538	4-9-3	54	1342
1585	1600	AGAGGTGAAGCGAAGT	552594	5-9-2	52	1342
1586	1601	CAGAGGTGAAGCGAAG	552539	4-9-3	50	1343
1586	1601	CAGAGGTGAAGCGAAG	552595	5-9-2	57	1343
1587	1602	GCAGAGGTGAAGCGAA	552540	4-9-3	60	1344
1587	1602	GCAGAGGTGAAGCGAA	552596	5-9-2	58	1344
1588	1603	TGCAGAGGTGAAGCGA	552541	4-9-3	68	1345
1588	1603	TGCAGAGGTGAAGCGA	552597	5-9-2	52	1345
1589	1604	GTGCAGAGGTGAAGCG	552542	4-9-3	63	1346
1589	1604	GTGCAGAGGTGAAGCG	552598	5-9-2	51	1346
1590	1605	CGTGCAGAGGTGAAGC	552543	4-9-3	44	1347
1590	1605	CGTGCAGAGGTGAAGC	552600	5-9-2	51	1347
1591	1606	ACGTGCAGAGGTGAAG	552544	4-9-3	45	1348
1591	1606	ACGTGCAGAGGTGAAG	552602	5-9-2	13	1348
1778	1793	TATGCCTACAGCCTCC	552545	4-9-3	42	230
1778	1793	TATGCCTACAGCCTCC	552604	5-9-2	42	230
1779	1794	TTATGCCTACAGCCTC	552546	4-9-3	46	231
1779	1794	TTATGCCTACAGCCTC	552606	5-9-2	42	231
1780	1795	TTTATGCCTACAGCCT	552547	4-9-3	38	232
1780	1795	TTTATGCCTACAGCCT	552608	5-9-2	37	232
1781	1796	ATTTATGCCTACAGCC	552548	4-9-3	49	233
1781	1796	ATTTATGCCTACAGCC	552610	5-9-2	41	233

1782	1797	AATTTATGCCTACAGC	552549	4-9-3	34	234
1782	1797	AATTTATGCCTACAGC	552612	5-9-2	23	234
1783	1798	CAATTTATGCCTACAG	552550	4-9-3	13	235
1783	1798	CAATTTATGCCTACAG	552614	5-9-2	11	235
1784	1799	CCAATTTATGCCTACA	552551	4-9-3	8	236
1784	1799	CCAATTTATGCCTACA	552616	5-9-2	6	236

Table 43

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3371

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	47	224
1780	1799	CCAATTTATGCCTACAGCCT	509934	5-10-5	67	50
58	77	GAACTGGAGCCACCAGCAGG	552007	6-10-4	53	83
58	77	GAACTGGAGCCACCAGCAGG	552039	7-10-3	74	83
253	272	AGAGAAGTCCACCACGAGTC	552008	6-10-4	47	103
253	272	AGAGAAGTCCACCACGAGTC	552040	7-10-3	57	103
411	430	TGAGGCATAGCAGCAGGATG	552009	6-10-4	70	136
411	430	TGAGGCATAGCAGCAGGATG	552041	7-10-3	65	136
412	431	ATGAGGCATAGCAGCAGGAT	552010	6-10-4	51	139
412	431	ATGAGGCATAGCAGCAGGAT	552042	7-10-3	59	139
413	432	GATGAGGCATAGCAGCAGGA	552011	6-10-4	47	142
413	432	GATGAGGCATAGCAGCAGGA	552043	7-10-3	36	142
414	433	AGATGAGGCATAGCAGCAGG	552012	6-10-4	62	20
414	433	AGATGAGGCATAGCAGCAGG	552044	7-10-3	82	20
415	434	AAGATGAGGCATAGCAGCAG	552013	6-10-4	72	22
415	434	AAGATGAGGCATAGCAGCAG	552045	7-10-3	62	22
416	435	GAAGATGAGGCATAGCAGCA	552014	6-10-4	73	24
416	435	GAAGATGAGGCATAGCAGCA	552046	7-10-3	74	24
417	436	AGAAGATGAGGCATAGCAGC	552015	6-10-4	66	26
417	436	AGAAGATGAGGCATAGCAGC	552047	7-10-3	60	26
418	437	AAGAAGATGAGGCATAGCAG	552016	6-10-4	67	28
418	437	AAGAAGATGAGGCATAGCAG	552048	7-10-3	60	28
687	706	CGAACCACTGAACAAATGGC	552017	6-10-4	72	39
687	706	CGAACCACTGAACAAATGGC	552049	7-10-3	68	39
1261	1280	TTCCGCAGTATGGATCGGCA	552018	6-10-4	89	719
1261	1280	TTCCGCAGTATGGATCGGCA	552050	7-10-3	86	719
1262	1281	GTTCCGCAGTATGGATCGGC	552019	6-10-4	87	212
1262	1281	GTTCCGCAGTATGGATCGGC	552051	7-10-3	86	212
1263	1282	AGTTCCGCAGTATGGATCGG	551986	4-10-6	64	720
1263	1282	AGTTCCGCAGTATGGATCGG	552020	6-10-4	86	720

1263	1282	AGTTCCGCAGTATGGATCGG	552052	7-10-3	87	720
1264	1283	GAGTTCCGCAGTATGGATCG	551987	4-10-6	76	721
1264	1283	GAGTTCCGCAGTATGGATCG	552021	6-10-4	84	721
1264	1283	GAGTTCCGCAGTATGGATCG	552053	7-10-3	75	721
1265	1284	GGAGTTCCGCAGTATGGATC	551988	4-10-6	5	1349
1265	1284	GGAGTTCCGCAGTATGGATC	552005	5-10-5	72	1349
1265	1284	GGAGTTCCGCAGTATGGATC	552022	6-10-4	80	1349
1265	1284	GGAGTTCCGCAGTATGGATC	552054	7-10-3	83	1349
1266	1285	AGGAGTTCCGCAGTATGGAT	551989	4-10-6	64	722
1266	1285	AGGAGTTCCGCAGTATGGAT	552023	6-10-4	78	722
1266	1285	AGGAGTTCCGCAGTATGGAT	552055	7-10-3	57	722
1577	1596	GTGAAGCGAAGTGCACACGG	551990	4-10-6	83	224
1577	1596	GTGAAGCGAAGTGCACACGG	552024	6-10-4	89	224
1577	1596	GTGAAGCGAAGTGCACACGG	552056	7-10-3	82	224
1578	1597	GGTGAAGCGAAGTGCACACG	551991	4-10-6	0	801
1578	1597	GGTGAAGCGAAGTGCACACG	552025	6-10-4	89	801
1578	1597	GGTGAAGCGAAGTGCACACG	552057	7-10-3	89	801
1579	1598	AGGTGAAGCGAAGTGCACAC	551992	4-10-6	67	802
1579	1598	AGGTGAAGCGAAGTGCACAC	552026	6-10-4	84	802
1579	1598	AGGTGAAGCGAAGTGCACAC	552058	7-10-3	82	802
1580	1599	GAGGTGAAGCGAAGTGCACA	551993	4-10-6	78	225
1580	1599	GAGGTGAAGCGAAGTGCACA	552027	6-10-4	85	225
1580	1599	GAGGTGAAGCGAAGTGCACA	552059	7-10-3	85	225
1581	1600	AGAGGTGAAGCGAAGTGCAC	551994	4-10-6	82	804
1581	1600	AGAGGTGAAGCGAAGTGCAC	552028	6-10-4	82	804
1581	1600	AGAGGTGAAGCGAAGTGCAC	552060	7-10-3	74	804
1582	1601	CAGAGGTGAAGCGAAGTGCA	551995	4-10-6	81	805
1582	1601	CAGAGGTGAAGCGAAGTGCA	552029	6-10-4	81	805
1582	1601	CAGAGGTGAAGCGAAGTGCA	552061	7-10-3	81	805
1583	1602	GCAGAGGTGAAGCGAAGTGC	551996	4-10-6	79	226
1583	1602	GCAGAGGTGAAGCGAAGTGC	552030	6-10-4	86	226
1583	1602	GCAGAGGTGAAGCGAAGTGC	552062	7-10-3	85	226
1584	1603	TGCAGAGGTGAAGCGAAGTG	551997	4-10-6	80	806
1584	1603	TGCAGAGGTGAAGCGAAGTG	552031	6-10-4	86	806
1585	1604	GTGCAGAGGTGAAGCGAAGT	551998	4-10-6	74	807
1585	1604	GTGCAGAGGTGAAGCGAAGT	552032	6-10-4	78	807
1586	1605	CGTGCAGAGGTGAAGCGAAG	551999	4-10-6	79	227
1586	1605	CGTGCAGAGGTGAAGCGAAG	552033	6-10-4	80	227
1587	1606	ACGTGCAGAGGTGAAGCGAA	552000	4-10-6	84	1350
1587	1606	ACGTGCAGAGGTGAAGCGAA	552006	5-10-5	86	1350
1587	1606	ACGTGCAGAGGTGAAGCGAA	552034	6-10-4	81	1350
1778	1797	AATTTATGCCTACAGCCTCC	552001	4-10-6	66	46
1778	1797	AATTTATGCCTACAGCCTCC	552035	6-10-4	55	46

1779	1798	CAATTTATGCCTACAGCCTC	552002	4-10-6	54	48
1779	1798	CAATTTATGCCTACAGCCTC	552036	6-10-4	58	48
1780	1799	CCAATTTATGCCTACAGCCT	552003	4-10-6	50	50
1780	1799	CCAATTTATGCCTACAGCCT	552037	6-10-4	43	50
1781	1800	ACCAATTTATGCCTACAGCC	552004	4-10-6	56	52
1781	1800	ACCAATTTATGCCTACAGCC	552038	6-10-4	66	52

Table 44

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3371

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	61	224
411	427	GGCATAGCAGCAGGATG	510100	3-10-4	66	17
58	74	CTGGAGCCACCAGCAGG	552168	3-9-5	64	1288
58	74	CTGGAGCCACCAGCAGG	552222	4-9-4	76	1288
59	75	ACTGGAGCCACCAGCAG	552169	3-9-5	65	1289
59	75	ACTGGAGCCACCAGCAG	552223	4-9-4	41	1289
60	76	AACTGGAGCCACCAGCA	552170	3-9-5	58	1290
60	76	AACTGGAGCCACCAGCA	552224	4-9-4	58	1290
61	77	GAACTGGAGCCACCAGC	552171	3-9-5	51	1291
61	77	GAACTGGAGCCACCAGC	552225	4-9-4	49	1291
253	269	GAAGTCCACCACGAGTC	552172	3-9-5	23	9
253	269	GAAGTCCACCACGAGTC	552226	4-9-4	36	9
254	270	AGAAGTCCACCACGAGT	552173	3-9-5	44	10
254	270	AGAAGTCCACCACGAGT	552227	4-9-4	20	10
255	271	GAGAAGTCCACCACGAG	552174	3-9-5	28	11
255	271	GAGAAGTCCACCACGAG	552228	4-9-4	29	11
256	272	AGAGAAGTCCACCACGA	552175	3-9-5	56	12
411	427	GGCATAGCAGCAGGATG	552176	3-9-5	66	17
412	428	AGGCATAGCAGCAGGAT	552177	3-9-5	53	18
413	429	GAGGCATAGCAGCAGGA	552178	3-9-5	57	19
414	430	TGAGGCATAGCAGCAGG	552179	3-9-5	56	21
415	431	ATGAGGCATAGCAGCAG	552180	3-9-5	51	23
416	432	GATGAGGCATAGCAGCA	552181	3-9-5	51	25
417	433	AGATGAGGCATAGCAGC	552182	3-9-5	63	27
418	434	AAGATGAGGCATAGCAG	552183	3-9-5	60	29
670	686	ACTAGTAAACTGAGCCA	552185	3-9-5	67	1292
671	687	CACTAGTAAACTGAGCC	552186	3-9-5	37	1293
672	688	GCACTAGTAAACTGAGC	552187	3-9-5	68	1294
687	703	ACCACTGAACAAATGGC	552188	3-9-5	71	40
688	704	AACCACTGAACAAATGG	552189	3-9-5	51	41

689	705	GAACCACTGAACAAATG	552190	3-9-5	47	42
690	706	CGAACCACTGAACAAAT	552191	3-9-5	50	43
1261	1277	CGCAGTATGGATCGGCA	552192	3-9-5	80	1295
1262	1278	CCGCAGTATGGATCGGC	552193	3-9-5	73	1296
1263	1279	TCCGCAGTATGGATCGG	552194	3-9-5	58	1297
1264	1280	TTCCGCAGTATGGATCG	552195	3-9-5	60	1298
1265	1281	GTTCCGCAGTATGGATC	552196	3-9-5	54	1299
1266	1282	AGTTCCGCAGTATGGAT	552197	3-9-5	64	1300
1267	1283	GAGTTCCGCAGTATGGA	552198	3-9-5	62	1301
1268	1284	GGAGTTCCGCAGTATGG	552199	3-9-5	57	1302
1269	1285	AGGAGTTCCGCAGTATG	552200	3-9-5	52	1303
1577	1593	AAGCGAAGTGCACACGG	552201	3-9-5	73	1304
1578	1594	GAAGCGAAGTGCACACG	552202	3-9-5	60	1305
1579	1595	TGAAGCGAAGTGCACAC	552203	3-9-5	60	1306
1580	1596	GTGAAGCGAAGTGCACA	552204	3-9-5	63	1307
1581	1597	GGTGAAGCGAAGTGCAC	552151	2-9-6	71	1308
1581	1597	GGTGAAGCGAAGTGCAC	552205	3-9-5	64	1308
1582	1598	AGGTGAAGCGAAGTGCA	552152	2-9-6	69	1309
1582	1598	AGGTGAAGCGAAGTGCA	552206	3-9-5	71	1309
1583	1599	GAGGTGAAGCGAAGTGC	552153	2-9-6	63	1310
1583	1599	GAGGTGAAGCGAAGTGC	552207	3-9-5	71	1310
1584	1600	AGAGGTGAAGCGAAGTG	552154	2-9-6	56	1311
1584	1600	AGAGGTGAAGCGAAGTG	552208	3-9-5	52	1311
1585	1601	CAGAGGTGAAGCGAAGT	552155	2-9-6	61	1312
1585	1601	CAGAGGTGAAGCGAAGT	552209	3-9-5	50	1312
1586	1602	GCAGAGGTGAAGCGAAG	552156	2-9-6	40	1313
1586	1602	GCAGAGGTGAAGCGAAG	552210	3-9-5	66	1313
1587	1603	TGCAGAGGTGAAGCGAA	552157	2-9-6	45	1314
1587	1603	TGCAGAGGTGAAGCGAA	552211	3-9-5	63	1314
1588	1604	GTGCAGAGGTGAAGCGA	552158	2-9-6	66	1315
1588	1604	GTGCAGAGGTGAAGCGA	552212	3-9-5	62	1315
1589	1605	CGTGCAGAGGTGAAGCG	552159	2-9-6	68	1316
1589	1605	CGTGCAGAGGTGAAGCG	552213	3-9-5	64	1316
1590	1606	ACGTGCAGAGGTGAAGC	552160	2-9-6	78	1317
1590	1606	ACGTGCAGAGGTGAAGC	552214	3-9-5	72	1317
1778	1794	TTATGCCTACAGCCTCC	552161	2-9-6	57	47
1778	1794	TTATGCCTACAGCCTCC	552215	3-9-5	54	47
1779	1795	TTTATGCCTACAGCCTC	552162	2-9-6	54	49
1779	1795	TTTATGCCTACAGCCTC	552216	3-9-5	49	49
1780	1796	ATTTATGCCTACAGCCT	552163	2-9-6	65	51
1780	1796	ATTTATGCCTACAGCCT	552217	3-9-5	50	51
1781	1797	AATTTATGCCTACAGCC	552164	2-9-6	48	53
1781	1797	AATTTATGCCTACAGCC	552218	3-9-5	39	53

1782	1798	CAATTTATGCCTACAGC	552165	2-9-6	46	54
1782	1798	CAATTTATGCCTACAGC	552219	3-9-5	41	54
1783	1799	CCAATTTATGCCTACAG	552166	2-9-6	42	55
1783	1799	CCAATTTATGCCTACAG	552220	3-9-5	32	55
1784	1800	ACCAATTTATGCCTACA	552167	2-9-6	47	56
1784	1800	ACCAATTTATGCCTACA	552221	3-9-5	33	56

Table 45

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3371

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	87 56	224
1780	1799	CCAATTTATGCCTACAGCCT	509934	5-10-5	56	50
411	427	GGCATAGCAGCAGGATG	510100	3-10-4	69	17
58	77	GAACTGGAGCCACCAGCAGG	552071	8-10-2	73	83
58	74	CTGGAGCCACCAGCAGG	552114	2-9-6	64	1288
59	75	ACTGGAGCCACCAGCAG	552115	2-9-6	61	1289
60	76	AACTGGAGCCACCAGCA	552116	2-9-6	53	1290
61	77	GAACTGGAGCCACCAGC	552117	2-9-6	69	1291
253	272	AGAGAAGTCCACCACGAGTC	552072	8-10-2	39	103
253	269	GAAGTCCACCACGAGTC	552118	2-9-6	49	9
254	270	AGAAGTCCACCACGAGT	552119	2-9-6	49	10
255	271	GAGAAGTCCACCACGAG	552120	2-9-6	21	11
256	272	AGAGAAGTCCACCACGA	552121	2-9-6	27	12
411	430	TGAGGCATAGCAGCAGGATG	552073	8-10-2	73	136
411	427	GGCATAGCAGCAGGATG	552122	2-9-6	48	17
412	431	ATGAGGCATAGCAGCAGGAT	552074	8-10-2	69	139
412	428	AGGCATAGCAGCAGGAT	552123	2-9-6	68	18
413	432	GATGAGGCATAGCAGCAGGA	552075	8-10-2	78	142
413	429	GAGGCATAGCAGCAGGA	552124	2-9-6	47	19
414	433	AGATGAGGCATAGCAGCAGG	552076	8-10-2	63	20
414	430	TGAGGCATAGCAGCAGG	552125	2-9-6	72	21
415	434	AAGATGAGGCATAGCAGCAG	552077	8-10-2	62	22
415	431	ATGAGGCATAGCAGCAG	552126	2-9-6	64	23
416	435	GAAGATGAGGCATAGCAGCA	552078	8-10-2	59	24
416	432	GATGAGGCATAGCAGCA	552127	2-9-6	65	25
417	436	AGAAGATGAGGCATAGCAGC	552079	8-10-2	80	26
417	433	AGATGAGGCATAGCAGC	552128	2-9-6	78	27
418	437	AAGAAGATGAGGCATAGCAG	552080	8-10-2	74	28
418	434	AAGATGAGGCATAGCAG	552129	2-9-6	68	29

457	473	ACGGGCAACATACCTTG	552130	2-9-6	46	33
670	686	ACTAGTAAACTGAGCCA	552131	2-9-6	61	1292
671	687	CACTAGTAAACTGAGCC	552132	2-9-6	66	1293
672	688	GCACTAGTAAACTGAGC	552133	2-9-6	78	1294
687	706	CGAACCACTGAACAAATGGC	552081	8-10-2	69	39
687	703	ACCACTGAACAAATGGC	552134	2-9-6	68	40
688	704	AACCACTGAACAAATGG	552135	2-9-6	59	41
689	705	GAACCACTGAACAAATG	552136	2-9-6	39	42
690	706	CGAACCACTGAACAAAT	552137	2-9-6	36	43
1261	1280	TTCCGCAGTATGGATCGGCA	552082	8-10-2	86	719
1261	1277	CGCAGTATGGATCGGCA	552138	2-9-6	80	1295
1262	1281	GTTCCGCAGTATGGATCGGC	552083	8-10-2	85	212
1262	1278	CCGCAGTATGGATCGGC	552139	2-9-6	80	1296
1263	1282	AGTTCCGCAGTATGGATCGG	552084	8-10-2	86	720
1263	1279	TCCGCAGTATGGATCGG	552140	2-9-6	70	1297
1264	1283	GAGTTCCGCAGTATGGATCG	552085	8-10-2	83	721
1264	1280	TTCCGCAGTATGGATCG	552141	2-9-6	72	1298
1265	1284	GGAGTTCCGCAGTATGGATC	552086	8-10-2	83	1349
1265	1281	GTTCCGCAGTATGGATC	552142	2-9-6	58	1299
1266	1285	AGGAGTTCCGCAGTATGGAT	552087	8-10-2	77	722
1266	1282	AGTTCCGCAGTATGGAT	552143	2-9-6	70	1300
1267	1283	GAGTTCCGCAGTATGGA	552144	2-9-6	66	1301
1268	1284	GGAGTTCCGCAGTATGG	552145	2-9-6	78	1302
1269	1285	AGGAGTTCCGCAGTATG	552146	2-9-6	63	1303
1577	1596	GTGAAGCGAAGTGCACACGG	552088	8-10-2	90	224
1577	1593	AAGCGAAGTGCACACGG	552147	2-9-6	80	1304
1578	1597	GGTGAAGCGAAGTGCACACG	552089	8-10-2	87	801
1578	1594	GAAGCGAAGTGCACACG	552148	2-9-6	74	1305
1579	1598	AGGTGAAGCGAAGTGCACAC	552090	8-10-2	85	802
1579	1595	TGAAGCGAAGTGCACAC	552149	2-9-6	79	1306
1580	1599	GAGGTGAAGCGAAGTGCACA	552091	8-10-2	84	225
1581	1600	AGAGGTGAAGCGAAGTGCAC	552092	8-10-2	86	804
1582	1601	CAGAGGTGAAGCGAAGTGCA	552093	8-10-2	82	805
1583	1602	GCAGAGGTGAAGCGAAGTGC	552094	8-10-2	84	226
1584	1603	TGCAGAGGTGAAGCGAAGTG	552063	7-10-3	79	806
1584	1603	TGCAGAGGTGAAGCGAAGTG	552095	8-10-2	85	806
1585	1604	GTGCAGAGGTGAAGCGAAGT	552064	7-10-3	83	807
1585	1604	GTGCAGAGGTGAAGCGAAGT	552096	8-10-2	88	807
1586	1605	CGTGCAGAGGTGAAGCGAAG	552065	7-10-3	86	227
1586	1605	CGTGCAGAGGTGAAGCGAAG	552097	8-10-2	90	227
1587	1606	ACGTGCAGAGGTGAAGCGAA	552066	7-10-3	35	1350
1587	1606	ACGTGCAGAGGTGAAGCGAA	552098	8-10-2	86	1350
1778	1797	AATTTATGCCTACAGCCTCC	552067	7-10-3	53	46

1778	1797	AATTTATGCCTACAGCCTCC	552099	8-10-2	66	46
1779	1798	CAATTTATGCCTACAGCCTC	552068	7-10-3	70	48
1779	1798	CAATTTATGCCTACAGCCTC	552100	8-10-2	67	48
1780	1799	CCAATTTATGCCTACAGCCT	552069	7-10-3	68	50
1780	1799	CCAATTTATGCCTACAGCCT	552101	8-10-2	65	50
1781	1800	ACCAATTTATGCCTACAGCC	552070	7-10-3	64	52
1781	1800	ACCAATTTATGCCTACAGCC	552102	8-10-2	54	52

Table 46

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3371

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	5-10-5	69 57	224
411	427	GGCATAGCAGCAGGATG	510100	3-10-4	59	17
58	74	CTGGAGCCACCAGCAGG	552330	6-9-2	50	1288
59	75	ACTGGAGCCACCAGCAG	552331	6-9-2	46	1289
60	76	AACTGGAGCCACCAGCA	552332	6-9-2	50	1290
61	77	GAACTGGAGCCACCAGC	552333	6-9-2	48	1291
253	269	GAAGTCCACCACGAGTC	552334	6-9-2	42	9
254	270	AGAAGTCCACCACGAGT	552335	6-9-2	30	10
255	271	GAGAAGTCCACCACGAG	552336	6-9-2	23	11
256	272	AGAGAAGTCCACCACGA	552337	6-9-2	42	12
411	427	GGCATAGCAGCAGGATG	552338	6-9-2	40	17
412	428	AGGCATAGCAGCAGGAT	552339	6-9-2	50	18
413	429	GAGGCATAGCAGCAGGA	552340	6-9-2	45	19
414	430	TGAGGCATAGCAGCAGG	552341	6-9-2	44	21
415	431	ATGAGGCATAGCAGCAG	552342	6-9-2	51	23
416	432	GATGAGGCATAGCAGCA	552343	6-9-2	44	25
417	433	AGATGAGGCATAGCAGC	552344	6-9-2	24	27
418	434	AAGATGAGGCATAGCAG	552345	6-9-2	41	29
457	473	ACGGGCAACATACCTTG	552346	6-9-2	0	33
670	686	ACTAGTAAACTGAGCCA	552347	6-9-2	75	1292
671	687	CACTAGTAAACTGAGCC	552348	6-9-2	72	1293
672	688	GCACTAGTAAACTGAGC	552349	6-9-2	65	1294
687	703	ACCACTGAACAAATGGC	552350	6-9-2	42	40
688	704	AACCACTGAACAAATGG	552351	6-9-2	45	41
689	705	GAACCACTGAACAAATG	552352	6-9-2	43	42
690	706	CGAACCACTGAACAAAT	552353	6-9-2	20	43
1261	1277	CGCAGTATGGATCGGCA	552354	6-9-2	70	1295
1262	1278	CCGCAGTATGGATCGGC	552355	6-9-2	66	1296

1263	1279	TCCGCAGTATGGATCGG	552356	6-9-2	62	1297
1264	1280	TTCCGCAGTATGGATCG	552357	6-9-2	53	1298
1265	1281	GTTCCGCAGTATGGATC	552358	6-9-2	57	1299
1266	1282	AGTTCCGCAGTATGGAT	552359	6-9-2	46	1300
1267	1283	GAGTTCCGCAGTATGGA	552360	6-9-2	45	1301
1268	1284	GGAGTTCCGCAGTATGG	552361	6-9-2	44	1302
1269	1285	AGGAGTTCCGCAGTATG	552308	5-9-3	38	1303
1269	1285	AGGAGTTCCGCAGTATG	552362	6-9-2	51	1303
1577	1593	AAGCGAAGTGCACACGG	552309	5-9-3	76	1304
1577	1593	AAGCGAAGTGCACACGG	552363	6-9-2	73	1304
1578	1594	GAAGCGAAGTGCACACG	552310	5-9-3	58	1305
1578	1594	GAAGCGAAGTGCACACG	552364	6-9-2	66	1305
1579	1595	TGAAGCGAAGTGCACAC	552311	5-9-3	38	1306
1579	1595	TGAAGCGAAGTGCACAC	552365	6-9-2	64	1306
1580	1596	GTGAAGCGAAGTGCACA	552150	2-9-6	68	1307
1580	1596	GTGAAGCGAAGTGCACA	552312	5-9-3	75	1307
1580	1596	GTGAAGCGAAGTGCACA	552366	6-9-2	55	1307
1581	1597	GGTGAAGCGAAGTGCAC	552313	5-9-3	66	1308
1581	1597	GGTGAAGCGAAGTGCAC	552367	6-9-2	67	1308
1582	1598	AGGTGAAGCGAAGTGCA	552314	5-9-3	56	1309
1582	1598	AGGTGAAGCGAAGTGCA	552368	6-9-2	41	1309
1583	1599	GAGGTGAAGCGAAGTGC	552315	5-9-3	46	1310
1583	1599	GAGGTGAAGCGAAGTGC	552369	6-9-2	52	1310
1584	1600	AGAGGTGAAGCGAAGTG	552316	5-9-3	55	1311
1584	1600	AGAGGTGAAGCGAAGTG	552370	6-9-2	35	1311
1585	1601	CAGAGGTGAAGCGAAGT	552317	5-9-3	53	1312
1585	1601	CAGAGGTGAAGCGAAGT	552371	6-9-2	58	1312
1586	1602	GCAGAGGTGAAGCGAAG	552318	5-9-3	59	1313
1586	1602	GCAGAGGTGAAGCGAAG	552372	6-9-2	68	1313
1587	1603	TGCAGAGGTGAAGCGAA	552319	5-9-3	56	1314
1587	1603	TGCAGAGGTGAAGCGAA	552373	6-9-2	63	1314
1588	1604	GTGCAGAGGTGAAGCGA	552320	5-9-3	62	1315
1588	1604	GTGCAGAGGTGAAGCGA	552374	6-9-2	70	1315
1589	1605	CGTGCAGAGGTGAAGCG	552321	5-9-3	63	1316
1589	1605	CGTGCAGAGGTGAAGCG	552375	6-9-2	64	1316
1590	1606	ACGTGCAGAGGTGAAGC	552322	5-9-3	52	1317
1590	1606	ACGTGCAGAGGTGAAGC	552376	6-9-2	58	1317
1778	1794	TTATGCCTACAGCCTCC	552323	5-9-3	45	47
1778	1794	TTATGCCTACAGCCTCC	552377	6-9-2	42	47
1779	1795	TTTATGCCTACAGCCTC	552324	5-9-3	49	49
1779	1795	TTTATGCCTACAGCCTC	552378	6-9-2	37	49
1780	1796	ATTTATGCCTACAGCCT	552325	5-9-3	48	51
1780	1796	ATTTATGCCTACAGCCT	552379	6-9-2	57	51

1781	1797	AATTTATGCCTACAGCC	552326	5-9-3	50	53
1781	1797	AATTTATGCCTACAGCC	552380	6-9-2	48	53
1782	1798	CAATTTATGCCTACAGC	552327	5-9-3	13	54
1782	1798	CAATTTATGCCTACAGC	552381	6-9-2	22	54
1783	1799	CCAATTTATGCCTACAG	552328	5-9-3	9	55
1783	1799	CCAATTTATGCCTACAG	552382	6-9-2	20	55
1784	1800	ACCAATTTATGCCTACA	552329	5-9-3	18	56
1784	1800	ACCAATTTATGCCTACA	552383	6-9-2	18	56

Example 15: Antisense inhibition of HBV viral mRNA in HepG2 cells by deoxy, MOE and (S)-cEt gapmers

Antisense oligonucleotides were designed targeting a HBV viral nucleic acid and were tested for their effects on HBV mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. ISIS 146786 and ISIS 509934, which were described in an earlier application (U.S. Provisional Application No. 61/478,040 filed on April 21, 2011), were also included in these studies for comparison. Cultured HepG2 cells at a density of 28,000 cells per well were transfected using LipofectAMINE2000® with 70 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe set RTS3370 (forward sequence CTTGGTCATGGGCCATCAG, designated herein as SEQ ID NO: 1; reverse sequence CGGCTAGGAGTTCCGCAGTA, designated herein as SEQ ID NO: 2; probe sequence TGCGTGGAACCTTTTCGGCTCC, designated herein as SEQ ID NO: 3) was used to measure mRNA levels. Levels were also measured using primer probe set RTS3371 (forward sequence CCAAACCTTCGGACGGAAA, designated herein as SEQ ID NO: 311; reverse sequence TGAGGCCCACTCCCATAGG, designated herein as SEQ ID NO: 312; probe sequence CCCATCATCCTGGGCTTTCGGAAAAT, designated herein as SEQ ID NO: 313). HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in Tables below were designed as deoxy, MOE and (S)-cEt gapmers. The gapmers are 16 nucleosides in length wherein the nucleoside have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; the number indicates the number of deoxynucleosides; and 'e' indicates a MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each oligonucleotide are 5-methylcytosines.

"Viral Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the viral gene sequence. "Viral Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted viral gene sequence. Each gapmer listed in the Tables is targeted to the viral genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. U95551.1). The potency of the newly designed oligonucleotides was compared with ISIS 146786, 509934, ISIS 509959, and ISIS 510100.

Table 47

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3370

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1780	1799	CCAATTTATGCCTACAGCCT	509934	eeeee-10-eeeee	30	50
58	73	TGGAGCCACCAGCAGG	552787	ekk-10-kke	57	1318
59	74	CTGGAGCCACCAGCAG	552788	ekk-10-kke	60	1319
60	75	ACTGGAGCCACCAGCA	552789	ekk-10-kke	67	1320
61	76	AACTGGAGCCACCAGC	552790	ekk-10-kke	67	1321
62	77	GAACTGGAGCCACCAG	552791	ekk-10-kke	65	86
245	260	CACGAGTCTAGACTCT	552792	ekk-10-kke	44	93
246	261	CCACGAGTCTAGACTC	552793	ekk-10-kke	0	95
250	265	TCCACCACGAGTCTAG	552794	ekk-10-kke	54	98
251	266	GTCCACCACGAGTCTA	552795	ekk-10-kke	55	100
252	267	AGTCCACCACGAGTCT	552796	ekk-10-kke	62	102
253	268	AAGTCCACCACGAGTC	552797	ekk-10-kke	59	104
254	269	GAAGTCCACCACGAGT	552798	ekk-10-kke	59	106
255	270	AGAAGTCCACCACGAG	552799	ekk-10-kke	58	109
256	271	GAGAAGTCCACCACGA	552800	ekk-10-kke	62	112
258	273	GAGAGAAGTCCACCAC	552801	ekk-10-kke	65	115
259	274	TGAGAGAAGTCCACCA	552802	ekk-10-kke	53	117
411	426	GCATAGCAGCAGGATG	552803	ekk-10-kke	67	137
412	427	GGCATAGCAGCAGGAT	552804	ekk-10-kke	75	140
413	428	AGGCATAGCAGCAGGA	552805	ekk-10-kke	72	143
414	429	GAGGCATAGCAGCAGG	552806	ekk-10-kke	64	145
415	430	TGAGGCATAGCAGCAG	552807	ekk-10-kke	68	147
416	431	ATGAGGCATAGCAGCA	552808	ekk-10-kke	65	149
417	432	GATGAGGCATAGCAGC	552809	ekk-10-kke	60	151
418	433	AGATGAGGCATAGCAG	552810	ekk-10-kke	59	153
419	434	AAGATGAGGCATAGCA	552811	ekk-10-kke	64	155
420	435	GAAGATGAGGCATAGC	552812	ekk-10-kke	69	157
421	436	AGAAGATGAGGCATAG	552813	ekk-10-kke	64	159
422	437	AAGAAGATGAGGCATA	552814	ekk-10-kke	62	161
457	472	CGGGCAACATACCTTG	552815	ekk-10-kke	61	167
458	473	ACGGGCAACATACCTT	552816	ekk-10-kke	63	168

639	654	GGCCCACTCCCATAGG	552817	ekk-10-kke	42	176
641	656	GAGGCCCACTCCCATA	552818	ekk-10-kke	44	177
642	657	TGAGGCCCACTCCCAT	552819	ekk-10-kke	56	178
643	658	CTGAGGCCCACTCCCA	552820	ekk-10-kke	59	179
670	685	CTAGTAAACTGAGCCA	552821	ekk-10-kke	76	181
671	686	ACTAGTAAACTGAGCC	552822	ekk-10-kke	77	1322
672	687	CACTAGTAAACTGAGC	552823	ekk-10-kke	73	1323
673	688	GCACTAGTAAACTGAG	552824	ekk-10-kke	73	1324
678	693	AAATGGCACTAGTAAA	552825	ekk-10-kke	51	1364
679	694	CAAATGGCACTAGTAA	552826	ekk-10-kke	55	1365
680	695	ACAAATGGCACTAGTA	552827	ekk-10-kke	67	1366
681	696	AACAAATGGCACTAGT	552828	ekk-10-kke	78	1367
682	697	GAACAAATGGCACTAG	552829	ekk-10-kke	72	1368
683	698	TGAACAAATGGCACTA	552830	ekk-10-kke	71	1369
684	699	CTGAACAAATGGCACT	552831	ekk-10-kke	69	1370
685	700	ACTGAACAAATGGCAC	552832	ekk-10-kke	67	1371
686	701	CACTGAACAAATGGCA	552833	ekk-10-kke	65	1372
687	702	CCACTGAACAAATGGC	552834	ekk-10-kke	78	188
688	703	ACCACTGAACAAATGG	552835	ekk-10-kke	70	190
689	704	AACCACTGAACAAATG	552836	ekk-10-kke	64	191
690	705	GAACCACTGAACAAAT	552837	ekk-10-kke	65	192
691	706	CGAACCACTGAACAAA	552838	ekk-10-kke	64	194
738	753	CCACATCATCCATATA	552839	ekk-10-kke	60	199
739	754	ACCACATCATCCATAT	552840	ekk-10-kke	35	201
1176	1191	CAGCAAACACTTGGCA	552841	ekk-10-kke	62	208
1177	1192	TCAGCAAACACTTGGC	552842	ekk-10-kke	67	209
1261	1276	GCAGTATGGATCGGCA	552843	ekk-10-kke	77	211
1262	1277	CGCAGTATGGATCGGC	552844	ekk-10-kke	81	1325
1263	1278	CCGCAGTATGGATCGG	552845	ekk-10-kke	63	1326
1264	1279	TCCGCAGTATGGATCG	552846	ekk-10-kke	79	1327
1265	1280	TTCCGCAGTATGGATC	552847	ekk-10-kke	47	1328
1266	1281	GTTCCGCAGTATGGAT	552848	ekk-10-kke	69	1329
1267	1282	AGTTCCGCAGTATGGA	552849	ekk-10-kke	59	1330
1268	1283	GAGTTCCGCAGTATGG	552850	ekk-10-kke	83	1331
1269	1284	GGAGTTCCGCAGTATG	552851	ekk-10-kke	90	1332
1270	1285	AGGAGTTCCGCAGTAT	552852	ekk-10-kke	89	1333
1577	1592	AGCGAAGTGCACACGG	552853	ekk-10-kke	83	1334
1578	1593	AAGCGAAGTGCACACG	552854	ekk-10-kke	80	1335
1579	1594	GAAGCGAAGTGCACAC	552855	ekk-10-kke	75	1336
1580	1595	TGAAGCGAAGTGCACA	552856	ekk-10-kke	69	1337
1581	1596	GTGAAGCGAAGTGCAC	552857	ekk-10-kke	68	1338
1582	1597	GGTGAAGCGAAGTGCA	552858	ekk-10-kke	79	1339
1583	1598	AGGTGAAGCGAAGTGC	552859	ekk-10-kke	79	1340

1584	1599	GAGGTGAAGCGAAGTG	552860	ekk-10-kke	71	1341
1585	1600	AGAGGTGAAGCGAAGT	552861	ekk-10-kke	68	1342
1586	1601	CAGAGGTGAAGCGAAG	552862	ekk-10-kke	65	1343
1587	1602	GCAGAGGTGAAGCGAA	552863	ekk-10-kke	70	1344
1588	1603	TGCAGAGGTGAAGCGA	552864	ekk-10-kke	71	1345

Table 48

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3371

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
58	73	TGGAGCCACCAGCAGG	552787	ekk-10-kke	53	1318
59	74	CTGGAGCCACCAGCAG	552788	ekk-10-kke	45	1319
60	75	ACTGGAGCCACCAGCA	552789	ekk-10-kke	75	1320
61	76	AACTGGAGCCACCAGC	552790	ekk-10-kke	68	1321
62	77	GAACTGGAGCCACCAG	552791	ekk-10-kke	51	86
245	260	CACGAGTCTAGACTCT	552792	ekk-10-kke	38	93
246	261	CCACGAGTCTAGACTC	552793	ekk-10-kke	0	95
250	265	TCCACCACGAGTCTAG	552794	ekk-10-kke	44	98
251	266	GTCCACCACGAGTCTA	552795	ekk-10-kke	56	100
252	267	AGTCCACCACGAGTCT	552796	ekk-10-kke	45	102
253	268	AAGTCCACCACGAGTC	552797	ekk-10-kke	46	104
254	269	GAAGTCCACCACGAGT	552798	ekk-10-kke	53	106
255	270	AGAAGTCCACCACGAG	552799	ekk-10-kke	48	109
256	271	GAGAAGTCCACCACGA	552800	ekk-10-kke	54	112
258	273	GAGAGAAGTCCACCAC	552801	ekk-10-kke	63	115
259	274	TGAGAGAAGTCCACCA	552802	ekk-10-kke	49	117
411	426	GCATAGCAGCAGGATG	552803	ekk-10-kke	71	137
412	427	GGCATAGCAGCAGGAT	552804	ekk-10-kke	64	140
413	428	AGGCATAGCAGCAGGA	552805	ekk-10-kke	70	143
414	429	GAGGCATAGCAGCAGG	552806	ekk-10-kke	67	145
415	430	TGAGGCATAGCAGCAG	552807	ekk-10-kke	61	147
416	431	ATGAGGCATAGCAGCA	552808	ekk-10-kke	83	149
417	432	GATGAGGCATAGCAGC	552809	ekk-10-kke	59	151
418	433	AGATGAGGCATAGCAG	552810	ekk-10-kke	56	153
419	434	AAGATGAGGCATAGCA	552811	ekk-10-kke	62	155
420	435	GAAGATGAGGCATAGC	552812	ekk-10-kke	66	157
421	436	AGAAGATGAGGCATAG	552813	ekk-10-kke	63	159
422	437	AAGAAGATGAGGCATA	552814	ekk-10-kke	65	161
457	472	CGGGCAACATACCTTG	552815	ekk-10-kke	63	167
458	473	ACGGGCAACATACCTT	552816	ekk-10-kke	88	168
639	654	GGCCCACTCCCATAGG	552817	ekk-10-kke	94	176

641	656	GAGGCCCACTCCCATA	552818	ekk-10-kke	82	177
642	657	TGAGGCCCACTCCCAT	552819	ekk-10-kke	80	178
643	658	CTGAGGCCCACTCCCA	552820	ekk-10-kke	84	179
670	685	CTAGTAAACTGAGCCA	552821	ekk-10-kke	71	181
671	686	ACTAGTAAACTGAGCC	552822	ekk-10-kke	85	1322
672	687	CACTAGTAAACTGAGC	552823	ekk-10-kke	71	1323
673	688	GCACTAGTAAACTGAG	552824	ekk-10-kke	81	1324
678	693	AAATGGCACTAGTAAA	552825	ekk-10-kke	51	1364
679	694	CAAATGGCACTAGTAA	552826	ekk-10-kke	64	1365
680	695	ACAAATGGCACTAGTA	552827	ekk-10-kke	61	1366
681	696	AACAAATGGCACTAGT	552828	ekk-10-kke	76	1367
682	697	GAACAAATGGCACTAG	552829	ekk-10-kke	61	1368
683	698	TGAACAAATGGCACTA	552830	ekk-10-kke	59	1369
684	699	CTGAACAAATGGCACT	552831	ekk-10-kke	58	1370
685	700	ACTGAACAAATGGCAC	552832	ekk-10-kke	64	1371
686	701	CACTGAACAAATGGCA	552833	ekk-10-kke	75	1372
687	702	CCACTGAACAAATGGC	552834	ekk-10-kke	84	188
688	703	ACCACTGAACAAATGG	552835	ekk-10-kke	57	190
689	704	AACCACTGAACAAATG	552836	ekk-10-kke	51	191
690	705	GAACCACTGAACAAAT	552837	ekk-10-kke	53	192
691	706	CGAACCACTGAACAAA	552838	ekk-10-kke	48	194
738	753	CCACATCATCCATATA	552839	ekk-10-kke	50	199
739	754	ACCACATCATCCATAT	552840	ekk-10-kke	54	201
1176	1191	CAGCAAACACTTGGCA	552841	ekk-10-kke	61	208
1177	1192	TCAGCAAACACTTGGC	552842	ekk-10-kke	71	209
1261	1276	GCAGTATGGATCGGCA	552843	ekk-10-kke	75	211
1262	1277	CGCAGTATGGATCGGC	552844	ekk-10-kke	78	1325
1263	1278	CCGCAGTATGGATCGG	552845	ekk-10-kke	52	1326
1264	1279	TCCGCAGTATGGATCG	552846	ekk-10-kke	76	1327
1265	1280	TTCCGCAGTATGGATC	552847	ekk-10-kke	61	1328
1266	1281	GTTCCGCAGTATGGAT	552848	ekk-10-kke	72	1329
1267	1282	AGTTCCGCAGTATGGA	552849	ekk-10-kke	87	1330
1268	1283	GAGTTCCGCAGTATGG	552850	ekk-10-kke	76	1331
1269	1284	GGAGTTCCGCAGTATG	552851	ekk-10-kke	76	1332
1270	1285	AGGAGTTCCGCAGTAT	552852	ekk-10-kke	79	1333
1577	1592	AGCGAAGTGCACACGG	552853	ekk-10-kke	82	1334
1578	1593	AAGCGAAGTGCACACG	552854	ekk-10-kke	85	1335
1579	1594	GAAGCGAAGTGCACAC	552855	ekk-10-kke	78	1336
1580	1595	TGAAGCGAAGTGCACA	552856	ekk-10-kke	77	1337
1581	1596	GTGAAGCGAAGTGCAC	552857	ekk-10-kke	75	1338
1582	1597	GGTGAAGCGAAGTGCA	552858	ekk-10-kke	75	1339
1583	1598	AGGTGAAGCGAAGTGC	552859	ekk-10-kke	79	1340
1584	1599	GAGGTGAAGCGAAGTG	552860	ekk-10-kke	71	1341

1585	1600	AGAGGTGAAGCGAAGT	552861	ekk-10-kke	74	1342
1586	1601	CAGAGGTGAAGCGAAG	552862	ekk-10-kke	66	1343
1587	1602	GCAGAGGTGAAGCGAA	552863	ekk-10-kke	70	1344
1588	1603	TGCAGAGGTGAAGCGA	552864	ekk-10-kke	73	1345

Table 49

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3371

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	eeeee-10-eeeee	60	224
58	73	TGGAGCCACCAGCAGG	552889	ek-10-keke	59	1318
59	74	CTGGAGCCACCAGCAG	552890	ek-10-keke	56	1319
60	75	ACTGGAGCCACCAGCA	552891	ek-10-keke	67	1320
61	76	AACTGGAGCCACCAGC	552892	ek-10-keke	65	1321
62	77	GAACTGGAGCCACCAG	552893	ek-10-keke	68	86
250	265	TCCACCACGAGTCTAG	552894	ek-10-keke	71	98
251	266	GTCCACCACGAGTCTA	552895	ek-10-keke	51	100
252	267	AGTCCACCACGAGTCT	552896	ek-10-keke	51	102
253	268	AAGTCCACCACGAGTC	552897	ek-10-keke	43	104
254	269	GAAGTCCACCACGAGT	552898	ek-10-keke	43	106
255	270	AGAAGTCCACCACGAG	552899	ek-10-keke	55	109
256	271	GAGAAGTCCACCACGA	552900	ek-10-keke	34	112
258	273	GAGAGAAGTCCACCAC	552901	ek-10-keke	42	115
259	274	TGAGAGAAGTCCACCA	552902	ek-10-keke	60	117
411	426	GCATAGCAGCAGGATG	552903	ek-10-keke	76	137
412	427	GGCATAGCAGCAGGAT	552904	ek-10-keke	74	140
413	428	AGGCATAGCAGCAGGA	552905	ek-10-keke	66	143
415	430	TGAGGCATAGCAGCAG	552907	ek-10-keke	69	147
416	431	ATGAGGCATAGCAGCA	552908	ek-10-keke	63	149
417	432	GATGAGGCATAGCAGC	552909	ek-10-keke	70	151
418	433	AGATGAGGCATAGCAG	552910	ek-10-keke	72	153
457	472	CGGGCAACATACCTTG	552911	ek-10-keke	72	167
458	473	ACGGGCAACATACCTT	552912	ek-10-keke	67	168
670	685	CTAGTAAACTGAGCCA	552913	ek-10-keke	74	181
682	697	GAACAAATGGCACTAG	552914	ek-10-keke	75	1368
684	699	CTGAACAAATGGCACT	552915	ek-10-keke	58	1370
686	701	CACTGAACAAATGGCA	552916	ek-10-keke	74	1372
687	702	CCACTGAACAAATGGC	552917	ek-10-keke	76	188
688	703	ACCACTGAACAAATGG	552918	ek-10-keke	75	190
689	704	AACCACTGAACAAATG	552919	ek-10-keke	55	191
690	705	GAACCACTGAACAAAT	552920	ek-10-keke	49	192

691	706	CGAACCACTGAACAAA	552921	ek-10-keke	45	194
1261	1276	GCAGTATGGATCGGCA	552922	ek-10-keke	83	211
1262	1277	CGCAGTATGGATCGGC	552923	ek-10-keke	83	1325
1263	1278	CCGCAGTATGGATCGG	552924	ek-10-keke	0	1326
1264	1279	TCCGCAGTATGGATCG	552925	ek-10-keke	85	1327
1265	1280	TTCCGCAGTATGGATC	552926	ek-10-keke	50	1328
1266	1281	GTTCCGCAGTATGGAT	552927	ek-10-keke	76	1329
1267	1282	AGTTCCGCAGTATGGA	552928	ek-10-keke	78	1330
1268	1283	GAGTTCCGCAGTATGG	552929	ek-10-keke	75	1331
1269	1284	GGAGTTCCGCAGTATG	552930	ek-10-keke	78	1332
1270	1285	AGGAGTTCCGCAGTAT	552931	ek-10-keke	74	1333
1577	1592	AGCGAAGTGCACACGG	552932	ek-10-keke	86	1334
1578	1593	AAGCGAAGTGCACACG	552933	ek-10-keke	82	1335
1579	1594	GAAGCGAAGTGCACAC	552934	ek-10-keke	74	1336
1580	1595	TGAAGCGAAGTGCACA	552935	ek-10-keke	76	1337
1581	1596	GTGAAGCGAAGTGCAC	552936	ek-10-keke	81	1338
1582	1597	GGTGAAGCGAAGTGCA	552937	ek-10-keke	80	1339
1583	1598	AGGTGAAGCGAAGTGC	552938	ek-10-keke	78	1340
1584	1599	GAGGTGAAGCGAAGTG	552939	ek-10-keke	75	1341
1585	1600	AGAGGTGAAGCGAAGT	552940	ek-10-keke	63	1342
1586	1601	CAGAGGTGAAGCGAAG	552941	ekk-10-kke	78	1343
1587	1602	GCAGAGGTGAAGCGAA	552942	ek-10-keke	80	1344
1589	1604	GTGCAGAGGTGAAGCG	552865	ekk-10-kke	67	1346
1590	1605	CGTGCAGAGGTGAAGC	552866	ekk-10-kke	68	1347
1778	1793	TATGCCTACAGCCTCC	552868	ekk-10-kke	55	230
1779	1794	TTATGCCTACAGCCTC	552869	ekk-10-kke	48	231
1780	1795	TTTATGCCTACAGCCT	552870	ekk-10-kke	55	232
1781	1796	ATTTATGCCTACAGCC	552871	ekk-10-kke	57	233
1782	1797	AATTTATGCCTACAGC	552872	ekk-10-kke	70	234
1783	1798	CAATTTATGCCTACAG	552873	ekk-10-kke	49	235
1784	1799	CCAATTTATGCCTACA	552874	ekk-10-kke	42	236
1785	1800	ACCAATTTATGCCTAC	552875	ekk-10-kke	41	237
1822	1837	GGCAGAGGTGAAAAAG	552876	ekk-10-kke	50	244
1823	1838	AGGCAGAGGTGAAAAA	552877	ek-10-keke	39	245
1824	1839	TAGGCAGAGGTGAAAA	552878	ekk-10-kke	31	247
1865	1880	AGCTTGGAGGCTTGAA	552879	ekk-10-kke	5	252
1866	1881	CAGCTTGGAGGCTTGA	552880	ekk-10-kke	5	254
1867	1882	ACAGCTTGGAGGCTTG	552881	ekk-10-kke	10	256
1868	1883	CACAGCTTGGAGGCTT	552882	ekk-10-kke	11	258
1869	1884	GCACAGCTTGGAGGCT	552883	ekk-10-kke	27	260
1870	1885	GGCACAGCTTGGAGGC	552884	ekk-10-kke	36	262
1871	1886	AGGCACAGCTTGGAGG	552885	ekk-10-kke	12	264
1872	1887	AAGGCACAGCTTGGAG	552886	ekk-10-kke	32	266

1874	1889	CCAAGGCACAGCTTGG	552888	ekk-10-kke	1	271

Table 50

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3371

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
1577	1596	GTGAAGCGAAGTGCACACGG	146786	eeeee-10-eeeee	59	224
58	73	TGGAGCCACCAGCAGG	552955	eee-10-kkk	60	1318
59	74	CTGGAGCCACCAGCAG	552956	eee-10-kkk	60	1319
60	75	ACTGGAGCCACCAGCA	552957	eee-10-kkk	64	1320
61	76	AACTGGAGCCACCAGC	552958	eee-10-kkk	56	1321
62	77	GAACTGGAGCCACCAG	552959	eee-10-kkk	59	86
250	265	TCCACCACGAGTCTAG	552960	eee-10-kkk	42	98
251	266	GTCCACCACGAGTCTA	552961	eee-10-kkk	41	100
252	267	AGTCCACCACGAGTCT	552962	eee-10-kkk	35	102
253	268	AAGTCCACCACGAGTC	552963	eee-10-kkk	19	104
254	269	GAAGTCCACCACGAGT	552964	eee-10-kkk	34	106
255	270	AGAAGTCCACCACGAG	552965	eee-10-kkk	42	109
256	271	GAGAAGTCCACCACGA	552966	eee-10-kkk	60	112
258	273	GAGAGAAGTCCACCAC	552967	eee-10-kkk	38	115
259	274	TGAGAGAAGTCCACCA	552968	eee-10-kkk	35	117
411	426	GCATAGCAGCAGGATG	552969	eee-10-kkk	67	137
412	427	GGCATAGCAGCAGGAT	552970	eee-10-kkk	56	140
413	428	AGGCATAGCAGCAGGA	552971	eee-10-kkk	69	143
414	429	GAGGCATAGCAGCAGG	552972	eee-10-kkk	75	145
415	430	TGAGGCATAGCAGCAG	552973	eee-10-kkk	59	145
416	431	ATGAGGCATAGCAGCA	552974	eee-10-kkk	71	149
417	432	GATGAGGCATAGCAGC	552975	eee-10-kkk	56	151
418	433	AGATGAGGCATAGCAG	552976	eee-10-kkk	50	153
457	472	CGGGCAACATACCTTG	552977	eee-10-kkk	56	167
458	473	ACGGGCAACATACCTT	552978	eee-10-kkk	43	168
670	685	CTAGTAAACTGAGCCA	552979	eee-10-kkk	71	181
682	697	GAACAAATGGCACTAG	552980	eee-10-kkk	80	1368
684	699	CTGAACAAATGGCACT	552981	eee-10-kkk	64	1370
686	701	CACTGAACAAATGGCA	552982	ek-10-keke	61	1372
687	702	CCACTGAACAAATGGC	552983	eee-10-kkk	77	188
688	703	ACCACTGAACAAATGG	552984	eee-10-kkk	65	190
689	704	AACCACTGAACAAATG	552985	eee-10-kkk	41	191
690	705	GAACCACTGAACAAAT	552986	eee-10-kkk	30	192
691	706	CGAACCACTGAACAAA	552987	eee-10-kkk	41	194
1261	1276	GCAGTATGGATCGGCA	552988	eee-10-kkk	74	211

1262	1277	CGCAGTATGGATCGGC	552989	eee-10-kkk	85	1325
1263	1278	CCGCAGTATGGATCGG	552990	eee-10-kkk	72	1326
1264	1279	TCCGCAGTATGGATCG	552991	eee-10-kkk	73	1327
1265	1280	TTCCGCAGTATGGATC	552992	eee-10-kkk	60	1328
1266	1281	GTTCCGCAGTATGGAT	552993	eee-10-kkk	52	1329
1267	1282	AGTTCCGCAGTATGGA	552994	eee-10-kkk	58	1330
1268	1283	GAGTTCCGCAGTATGG	552995	eee-10-kkk	70	1331
1269	1284	GGAGTTCCGCAGTATG	552996	eee-10-kkk	74	1332
1270	1285	AGGAGTTCCGCAGTAT	552997	eee-10-kkk	59	1333
1577	1592	AGCGAAGTGCACACGG	552998	eee-10-kkk	82	1334
1578	1593	AAGCGAAGTGCACACG	552999	eee-10-kkk	70	1335
1579	1594	GAAGCGAAGTGCACAC	553000	eee-10-kkk	67	1336
1580	1595	TGAAGCGAAGTGCACA	553001	eee-10-kkk	67	1337
1581	1596	GTGAAGCGAAGTGCAC	553002	eee-10-kkk	74	1338
1582	1597	GGTGAAGCGAAGTGCA	553003	eee-10-kkk	72	1339
1583	1598	AGGTGAAGCGAAGTGC	553004	eee-10-kkk	73	1340
1584	1599	GAGGTGAAGCGAAGTG	553005	eee-10-kkk	67	1341
1585	1600	AGAGGTGAAGCGAAGT	553006	eee-10-kkk	69	1342
1586	1601	CAGAGGTGAAGCGAAG	553007	eee-10-kkk	60	1343
1587	1602	GCAGAGGTGAAGCGAA	553008	eee-10-kkk	71	1344
1588	1603	TGCAGAGGTGAAGCGA	552943	ek-10-keke	77	1345
1588	1603	TGCAGAGGTGAAGCGA	553009	eee-10-kkk	78	1345
1589	1604	GTGCAGAGGTGAAGCG	552944	ek-10-keke	74	1346
1589	1604	GTGCAGAGGTGAAGCG	553010	eee-10-kkk	78	1346
1590	1605	CGTGCAGAGGTGAAGC	552945	ek-10-keke	76	1347
1590	1605	CGTGCAGAGGTGAAGC	553011	eee-10-kkk	72	1347
1591	1606	ACGTGCAGAGGTGAAG	552946	ek-10-keke	71	1348
1591	1606	ACGTGCAGAGGTGAAG	553012	eee-10-kkk	74	1348
1778	1793	TATGCCTACAGCCTCC	552947	ek-10-keke	54	230
1778	1793	TATGCCTACAGCCTCC	553013	eee-10-kkk	39	230
1779	1794	TTATGCCTACAGCCTC	552948	ek-10-keke	50	231
1779	1794	TTATGCCTACAGCCTC	553014	eee-10-kkk	37	231
1780	1795	TTTATGCCTACAGCCT	552949	ek-10-keke	8	232
1780	1795	TTTATGCCTACAGCCT	553015	eee-10-kkk	45	232
1781	1796	ATTTATGCCTACAGCC	552950	ek-10-keke	44	233
1781	1796	ATTTATGCCTACAGCC	553016	eee-10-kkk	47	233
1782	1797	AATTTATGCCTACAGC	552951	ek-10-keke	60	234
1782	1797	AATTTATGCCTACAGC	553017	eee-10-kkk	47	234
1783	1798	CAATTTATGCCTACAG	552952	ek-10-keke	35	235
1783	1798	CAATTTATGCCTACAG	553018	eee-10-kkk	30	235
1784	1799	CCAATTTATGCCTACA	552953	ek-10-keke	37	236
1784	1799	CCAATTTATGCCTACA	553019	eee-10-kkk	37	236
1785	1800	ACCAATTTATGCCTAC	552954	ek-10-keke	40	237

1785	1800	ACCAATTTATGCCTAC	553020	eee-10-kkk	24	237

Table 51

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3370

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
58	73	TGGAGCCACCAGCAGG	552889	ek-10-keke	42	1318
59	74	CTGGAGCCACCAGCAG	552890	ek-10-keke	56	1319
60	75	ACTGGAGCCACCAGCA	552891	ek-10-keke	55	1320
61	76	AACTGGAGCCACCAGC	552892	ek-10-keke	53	1321
62	77	GAACTGGAGCCACCAG	552893	ek-10-keke	56	86
250	265	TCCACCACGAGTCTAG	552894	ek-10-keke	53	98
251	266	GTCCACCACGAGTCTA	552895	ek-10-keke	38	100
252	267	AGTCCACCACGAGTCT	552896	ek-10-keke	43	102
253	268	AAGTCCACCACGAGTC	552897	ek-10-keke	40	104
254	269	GAAGTCCACCACGAGT	552898	ek-10-keke	50	106
255	270	AGAAGTCCACCACGAG	552899	ek-10-keke	37	109
256	271	GAGAAGTCCACCACGA	552900	ek-10-keke	43	112
258	273	GAGAGAAGTCCACCAC	552901	ek-10-keke	56	115
259	274	TGAGAGAAGTCCACCA	552902	ek-10-keke	43	117
411	426	GCATAGCAGCAGGATG	552903	ek-10-keke	78	137
412	427	GGCATAGCAGCAGGAT	552904	ek-10-keke	75	140
413	428	AGGCATAGCAGCAGGA	552905	ek-10-keke	52	143
415	430	TGAGGCATAGCAGCAG	552907	ek-10-keke	75	147
416	431	ATGAGGCATAGCAGCA	552908	ek-10-keke	57	149
417	432	GATGAGGCATAGCAGC	552909	ek-10-keke	66	151
418	433	AGATGAGGCATAGCAG	552910	ek-10-keke	60	153
457	472	CGGGCAACATACCTTG	552911	ek-10-keke	65	167
458	473	ACGGGCAACATACCTT	552912	ek-10-keke	37	168
670	685	CTAGTAAACTGAGCCA	552913	ek-10-keke	76	181
682	697	GAACAAATGGCACTAG	552914	ek-10-keke	79	1368
684	699	CTGAACAAATGGCACT	552915	ek-10-keke	71	1370
686	701	CACTGAACAAATGGCA	552916	ek-10-keke	82	1372
687	702	CCACTGAACAAATGGC	552917	ek-10-keke	78	188
688	703	ACCACTGAACAAATGG	552918	ek-10-keke	64	190
689	704	AACCACTGAACAAATG	552919	ek-10-keke	38	191
690	705	GAACCACTGAACAAAT	552920	ek-10-keke	43	192
691	706	CGAACCACTGAACAAA	552921	ek-10-keke	49	194
1261	1276	GCAGTATGGATCGGCA	552922	ek-10-keke	90	211
1262	1277	CGCAGTATGGATCGGC	552923	ek-10-keke	92	1325
1263	1278	CCGCAGTATGGATCGG	552924	ek-10-keke	30	1326

1264	1279	TCCGCAGTATGGATCG	552925	ek-10-keke	81	1327
1265	1280	TTCCGCAGTATGGATC	552926	ek-10-keke	39	1328
1266	1281	GTTCCGCAGTATGGAT	552927	ek-10-keke	53	1329
1267	1282	AGTTCCGCAGTATGGA	552928	ek-10-keke	48	1330
1268	1283	GAGTTCCGCAGTATGG	552929	ek-10-keke	68	1331
1269	1284	GGAGTTCCGCAGTATG	552930	ek-10-keke	87	1332
1270	1285	AGGAGTTCCGCAGTAT	552931	ek-10-keke	87	1333
1577	1592	AGCGAAGTGCACACGG	552932	ek-10-keke	88	1334
1578	1593	AAGCGAAGTGCACACG	552933	ek-10-keke	75	1335
1579	1594	GAAGCGAAGTGCACAC	552934	ek-10-keke	76	1336
1580	1595	TGAAGCGAAGTGCACA	552935	ek-10-keke	71	1337
1581	1596	GTGAAGCGAAGTGCAC	552936	ek-10-keke	80	1338
1582	1597	GGTGAAGCGAAGTGCA	552937	ek-10-keke	81	1339
1583	1598	AGGTGAAGCGAAGTGC	552938	ek-10-keke	85	1340
1584	1599	GAGGTGAAGCGAAGTG	552939	ek-10-keke	82	1341
1585	1600	AGAGGTGAAGCGAAGT	552940	ek-10-keke	76	1342
1586	1601	CAGAGGTGAAGCGAAG	552941	ekk-10-kke	72	1343
1587	1602	GCAGAGGTGAAGCGAA	552942	ek-10-keke	85	1344
1589	1604	GTGCAGAGGTGAAGCG	552865	ekk-10-kke	70	1346
1590	1605	CGTGCAGAGGTGAAGC	552866	ekk-10-kke	65	1347
1778	1793	TATGCCTACAGCCTCC	552868	ekk-10-kke	36	230
1779	1794	TTATGCCTACAGCCTC	552869	ekk-10-kke	23	231
1780	1795	TTTATGCCTACAGCCT	552870	ekk-10-kke	49	232
1781	1796	ATTTATGCCTACAGCC	552871	ekk-10-kke	46	233
1782	1797	AATTTATGCCTACAGC	552872	ekk-10-kke	73	234
1783	1798	CAATTTATGCCTACAG	552873	ekk-10-kke	41	235
1784	1799	CCAATTTATGCCTACA	552874	ekk-10-kke	18	236
1785	1800	ACCAATTTATGCCTAC	552875	ekk-10-kke	0	237
1822	1837	GGCAGAGGTGAAAAAG	552876	ekk-10-kke	49	244
1823	1838	AGGCAGAGGTGAAAAA	552877	ek-10-keke	37	245
1824	1839	TAGGCAGAGGTGAAAA	552878	ekk-10-kke	28	247
1865	1880	AGCTTGGAGGCTTGAA	552879	ekk-10-kke	0	252
1866	1881	CAGCTTGGAGGCTTGA	552880	ekk-10-kke	12	254
1867	1882	ACAGCTTGGAGGCTTG	552881	ekk-10-kke	0	256
1868	1883	CACAGCTTGGAGGCTT	552882	ekk-10-kke	0	258
1869	1884	GCACAGCTTGGAGGCT	552883	ekk-10-kke	12	260
1870	1885	GGCACAGCTTGGAGGC	552884	ekk-10-kke	39	262
1871	1886	AGGCACAGCTTGGAGG	552885	ekk-10-kke	37	264
1872	1887	AAGGCACAGCTTGGAG	552886	ekk-10-kke	15	266
1874	1889	CCAAGGCACAGCTTGG	552888	ekk-10-kke	0	271

Table 52

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3370

Viral Target Start Site	Viral Target Stop Site	Sequence	ISIS No	Motif	% inhibition	SEQ ID NO
58	73	TGGAGCCACCAGCAGG	552955	eee-10-kkk	67	1318
59	74	CTGGAGCCACCAGCAG	552956	eee-10-kkk	60	1319
60	75	ACTGGAGCCACCAGCA	552957	eee-10-kkk	73	1320
61	76	AACTGGAGCCACCAGC	552958	eee-10-kkk	63	1321
62	77	GAACTGGAGCCACCAG	552959	eee-10-kkk	58	86
250	265	TCCACCACGAGTCTAG	552960	eee-10-kkk	67	98
251	266	GTCCACCACGAGTCTA	552961	eee-10-kkk	78	100
252	267	AGTCCACCACGAGTCT	552962	eee-10-kkk	29	102
253	268	AAGTCCACCACGAGTC	552963	eee-10-kkk	25	104
254	269	GAAGTCCACCACGAGT	552964	eee-10-kkk	33	106
255	270	AGAAGTCCACCACGAG	552965	eee-10-kkk	55	109
256	271	GAGAAGTCCACCACGA	552966	eee-10-kkk	71	112
258	273	GAGAGAAGTCCACCAC	552967	eee-10-kkk	23	115
259	274	TGAGAGAAGTCCACCA	552968	eee-10-kkk	41	117
411	426	GCATAGCAGCAGGATG	552969	eee-10-kkk	76	137
412	427	GGCATAGCAGCAGGAT	552970	eee-10-kkk	44	140
413	428	AGGCATAGCAGCAGGA	552971	eee-10-kkk	77	143
414	429	GAGGCATAGCAGCAGG	552972	eee-10-kkk	74	145
415	430	TGAGGCATAGCAGCAG	552973	eee-10-kkk	61	145
416	431	ATGAGGCATAGCAGCA	552974	eee-10-kkk	73	149
417	432	GATGAGGCATAGCAGC	552975	eee-10-kkk	66	151
418	433	AGATGAGGCATAGCAG	552976	eee-10-kkk	70	153
457	472	CGGGCAACATACCTTG	552977	eee-10-kkk	65	167
458	473	ACGGGCAACATACCTT	552978	eee-10-kkk	40	168
670	685	CTAGTAAACTGAGCCA	552979	eee-10-kkk	79	181
682	697	GAACAAATGGCACTAG	552980	eee-10-kkk	81	64
684	699	CTGAACAAATGGCACT	552981	eee-10-kkk	74	66
686	701	CACTGAACAAATGGCA	552982	ek-10-keke	52	68
687	702	CCACTGAACAAATGGC	552983	eee-10-kkk	78	188
688	703	ACCACTGAACAAATGG	552984	eee-10-kkk	71	190
689	704	AACCACTGAACAAATG	552985	eee-10-kkk	38	191
690	705	GAACCACTGAACAAAT	552986	eee-10-kkk	48	192
691	706	CGAACCACTGAACAAA	552987	eee-10-kkk	54	194
1261	1276	GCAGTATGGATCGGCA	552988	eee-10-kkk	85	211
1262	1277	CGCAGTATGGATCGGC	552989	eee-10-kkk	84	1325
1263	1278	CCGCAGTATGGATCGG	552990	eee-10-kkk	79	1326
1264	1279	TCCGCAGTATGGATCG	552991	eee-10-kkk	53	1327

1265	1280	TTCCGCAGTATGGATC	552992	eee-10-kkk	68	1328
1266	1281	GTTCCGCAGTATGGAT	552993	eee-10-kkk	67	1329
1267	1282	AGTTCCGCAGTATGGA	552994	eee-10-kkk	69	1330
1268	1283	GAGTTCCGCAGTATGG	552995	eee-10-kkk	62	1331
1269	1284	GGAGTTCCGCAGTATG	552996	eee-10-kkk	82	1332
1270	1285	AGGAGTTCCGCAGTAT	552997	eee-10-kkk	58	1333
1577	1592	AGCGAAGTGCACACGG	552998	eee-10-kkk	86	1334
1578	1593	AAGCGAAGTGCACACG	552999	eee-10-kkk	63	1335
1579	1594	GAAGCGAAGTGCACAC	553000	eee-10-kkk	67	1336
1580	1595	TGAAGCGAAGTGCACA	553001	eee-10-kkk	70	1337
1581	1596	GTGAAGCGAAGTGCAC	553002	eee-10-kkk	84	1338
1582	1597	GGTGAAGCGAAGTGCA	553003	eee-10-kkk	83	1339
1583	1598	AGGTGAAGCGAAGTGC	553004	eee-10-kkk	68	1340
1584	1599	GAGGTGAAGCGAAGTG	553005	eee-10-kkk	57	1341
1585	1600	AGAGGTGAAGCGAAGT	553006	eee-10-kkk	74	1342
1586	1601	CAGAGGTGAAGCGAAG	553007	eee-10-kkk	62	1343
1587	1602	GCAGAGGTGAAGCGAA	553008	eee-10-kkk	50	1344
1588	1603	TGCAGAGGTGAAGCGA	552943	ek-10-keke	86	1345
1588	1603	TGCAGAGGTGAAGCGA	553009	eee-10-kkk	79	1345
1589	1604	GTGCAGAGGTGAAGCG	552944	ek-10-keke	83	1346
1589	1604	GTGCAGAGGTGAAGCG	553010	eee-10-kkk	74	1346
1590	1605	CGTGCAGAGGTGAAGC	552945	ek-10-keke	79	1347
1590	1605	CGTGCAGAGGTGAAGC	553011	eee-10-kkk	60	1347
1591	1606	ACGTGCAGAGGTGAAG	552946	ek-10-keke	68	1348
1591	1606	ACGTGCAGAGGTGAAG	553012	eee-10-kkk	78	1348
1778	1793	TATGCCTACAGCCTCC	552947	ek-10-keke	51	230
1778	1793	TATGCCTACAGCCTCC	553013	eee-10-kkk	45	230
1779	1794	TTATGCCTACAGCCTC	552948	ek-10-keke	56	231
1779	1794	TTATGCCTACAGCCTC	553014	eee-10-kkk	53	231
1780	1795	TTTATGCCTACAGCCT	552949	ek-10-keke	1	232
1780	1795	TTTATGCCTACAGCCT	553015	eee-10-kkk	55	232
1781	1796	ATTTATGCCTACAGCC	552950	ek-10-keke	52	233
1781	1796	ATTTATGCCTACAGCC	553016	eee-10-kkk	65	233
1782	1797	AATTTATGCCTACAGC	552951	ek-10-keke	59	234
1782	1797	AATTTATGCCTACAGC	553017	eee-10-kkk	36	234
1783	1798	CAATTTATGCCTACAG	552952	ek-10-keke	34	235
1783	1798	CAATTTATGCCTACAG	553018	eee-10-kkk	20	235
1784	1799	CCAATTTATGCCTACA	552953	ek-10-keke	55	236
1784	1799	CCAATTTATGCCTACA	553019	eee-10-kkk	34	236
1785	1800	ACCAATTTATGCCTAC	552954	ek-10-keke	51	237
1785	1800	ACCAATTTATGCCTAC	553020	eee-10-kkk	28	237

Example 16: Dose-dependent antisense inhibition of HBV mRNA in HepG2 cells by MOE gapmers

Antisense oligonucleotides from the study described in Example 14 exhibiting *in vitro* inhibition of HBV mRNA were selected and tested at various doses in HepG2 cells. Cells were plated at a density of 28,000 cells per well and transfected using LipofectAMINE2000® with 9.26 nM, 27.78 nM, 83.33 nM, and 250.00 nM concentrations of antisense oligonucleotide, as specified in Table 53. After a treatment period of approximately 16 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. HBV primer probe set RTS3371 was used to measure mRNA levels. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells.

As illustrated in Table 53, HBV mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells. 'n/a' indicates that the data for that dosage is not available.

Table 53

Dose-dependent antisense inhibition of human HBV in HepG2 cells

ISIS No	9.2593 nM	27.7778 nM	83.3333 nM	250.0 nM
146786	10	43	74	89
509934	12	31	52	79
509959	4	24	49	67
510100	11	28	60	77
510124	3	11	13	41
551926	1	26	51	76
551958	15	17	56	82
551987	4	40	65	81
551990	7	55	78	91
551993	15	30	70	80
551994	0	30	39	58
551995	6	41	73	85
551996	13	47	71	85
551997	16	38	68	89
551998	4	36	69	85
551999	10	31	67	86
552000	0	17	61	78
552006	6	37	74	89
552009	1	5	39	60
552013	0	28	3	72
552014	0	26	32	77
552018	6	27	63	81
552019	15	34	65	90

552020	2	35	65	91
552021	4	11	53	82
552022	6	35	57	79
552023	11	33	59	81
552024	15	43	69	91
552025	17	35	69	87
552026	14	26	66	86
552027	3	46	62	88
552028	9	43	58	78
552029	8	40	72	89
552030	18	48	77	92
552031	0	38	66	89
552032	42	48	80	88
552033	2	40	64	84
552034	6	40	70	81
552039	2	33	56	83
552044	19	30	63	84
552046	4	21	47	77
552050	15	44	70	92
552051	8	33	69	90
552052	17	38	71	91
552053	0	40	59	86
552054	7	15	58	75
552056	19	62	86	92
552057	11	33	69	86
552058	30	55	79	90
552059	11	25	69	90
552060	9	32	61	86
552061	6	40	69	88
552062	22	48	75	89
552064	23	49	69	90
552065	10	8	69	86
552069	11	4	28	60
552073	9	31	62	78
552075	21	18	33	65
552077	0	17	40	72
552079	1	12	44	70
552080	3	12	34	69
552082	13	29	66	87
552083	24	54	69	88
552084	10	25	48	82
552085	28	35	64	85
552086	0	24	65	84

552088	33	53	77	93
552089	0	41	69	92
552090	17	35	70	87
552091	13	31	69	89
552092	6	23	66	89
552093	0	17	61	89
552094	12	38	65	88
552095	20	42	73	88
552096	n/a	39	66	91
552097	24	43	67	88
552098	0	24	56	85
552101	3	13	28	61
552147	11	27	58	80
552160	20	25	69	89
552163	0	21	22	53
552176	16	11	40	66
552192	7	38	78	89
552222	0	24	65	79
552247	0	38	69	86
552255	5	27	69	81
552301	5	38	65	86
552309	8	26	62	85
552312	0	4	32	62
552347	2	15	38	75
552348	12	40	42	65
552354	10	35	44	76
552361	2	25	55	74
552363	20	36	54	76
552374	7	4	38	76
552379	0	12	24	46
552403	8	27	54	76
552408	2	25	44	77
552409	6	31	56	80
552418	0	30	72	84
552420	9	34	53	81
552442	4	23	46	56
552466	0	23	56	79
552474	11	34	66	87
552477	11	22	44	64
552530	25	37	73	87
552559	9	13	29	51

Example 17: Dose-dependent antisense inhibition of HBV mRNA in HepG2 cells by deoxy, MOE and (S)-cEt gapmers

Antisense oligonucleotides from the study described in Example 15 exhibiting *in vitro* inhibition of HBV mRNA were selected and tested at various doses in HepG2 cells. Cells were plated at a density of 28,000 cells per well and transfected using LipofectAMINE2000® with 9.26 nM, 27.78 nM, 83.33 nM, and 250.00 nM concentrations of antisense oligonucleotide, as specified in Table 54. After a treatment period of approximately 16 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. HBV primer probe set RTS3371 was used to measure mRNA levels. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells.

As illustrated in Table 54, HBV mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 54

Dose-dependent antisense inhibition of human HBV in HepG2 cells

ISIS No	9.2593 nM	27.7778 nM	83.3333 nM	250.0 nM
146786	10	43	74	89
552808	13	14	55	70
552816	38	73	87	92
552818	29	63	87	85
552820	58	83	90	90
552821	33	49	71	88
552822	24	55	74	88
552824	8	24	65	87
552834	11	28	68	89
552849	12	25	73	84
552851	13	42	74	89
552852	4	35	70	87
552853	19	52	86	93
552854	28	57	80	89
552916	5	26	64	82
552922	25	44	77	89
552923	22	49	82	91
552925	33	56	80	92
552930	12	49	79	89
552931	12	40	62	82
552932	24	62	84	91
552933	20	40	75	89

552936	18	36	75	88
552937	22	51	82	88
552938	12	36	67	80
552939	17	40	65	79
552942	21	48	74	88
552943	5	39	70	85
552944	14	33	70	77
552980	15	40	69	86
552988	4	36	58	84
552989	0	50	74	81
552996	0	25	53	72
552998	17	49	79	90
553002	0	32	68	86
553003	15	42	67	88

Example 18: Antisense inhibition of HBV viral mRNA in HepG2 cells by deoxy, MOE and (S)-cEt gapmers

Additional antisense oligonucleotides were designed targeting a HBV viral nucleic acid and were tested for their effects on HBV mRNA in vitro. ISIS 5808 and ISIS 9591, disclosed in US5985662, as well as ISIS 146781, ISIS 146786, 524518, ISIS 552859, and ISIS 552870 were also included in these studies for comparison and are distinguished with an asterisk. Cultured HepG2 cells at a density of 28,000 cells per well were transfected using LipofectAMINE2000® with 100 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe sets RTS3370 and RTS3371 and were used to separately measure mRNA levels. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in Table below were designed as MOE gapmers or deoxy, MOE and (S)-cEt gapmers. The 5-10-5 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleosides each. The deoxy, MOE and (S)-cEt gapmers are 16 nucleosides in length wherein the nucleoside have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; the number indicates the number of deoxynucleosides; otherwise, 'd' indicates a deoxynucleoside; and 'e' indicates a MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each oligonucleotide are 5-methylcytosines.

"Viral Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the viral gene sequence. "Viral Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted viral gene sequence. Each gapmer listed in Table 55 is targeted to the viral genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. U95551.1).

Table 55

Inhibition of viral HBV mRNA levels by chimeric antisense oligonucleotides measured with RTS3370 or RTS3371

Viral Target Start Site	Viral Target Stop Site	ISIS No	Motif	% inhibition (RTS3370)	% inhibition (RTS3371)	Sequence	SEQ ID NO
156	176	5808*	Uniform deoxy	57	64	CCTGATGTGATGTTCTCCATG	1373
303	322	524518*	eeeee-10-eeeee	62	72	GGGACTGCGAATTTTGGCCA	428
376	395	146781*	eeeee-10-eeeee	72	93	AAACGCCGCAGACACATCCA	1374
380	399	582665	eeeee-10-eeeee	57	59	GATAAAACGCCGCAGACACA	1375
382	401	582666	eeeee-10-eeeee	49	92	ATGATAAAACGCCGCAGACA	1376
411	426	566831	kdkdk-9-ee	96	73	GCATAGCAGCAGGATG	137
411	427	577123	eekk-9-ekee	84	96	GGCATAGCAGCAGGATG	17
411	427	577124	kdkdk-8-eeee	92	96	GGCATAGCAGCAGGATG	17
411	426	577126	kkk-8-eeeee	87	90	GCATAGCAGCAGGATG	137
413	428	566830	kdkdk-9-ee	93	95	AGGCATAGCAGCAGGA	143
415	430	577130	eek-10-kke	87	94	TGAGGCATAGCAGCAG	147
415	430	577131	kdkdk-9-ee	83	93	TGAGGCATAGCAGCAG	147
1263	1278	566828	kdkdk-9-ee	97	90	CCGCAGTATGGATCGG	1236
1577	1596	146786*	eeeee-10-eeeee	93	71	GTGAAGCGAAGTGCACACGG	224
1577	1592	566829	kdkdk-9-ee	98	84	AGCGAAGTGCACACGG	1334
1577	1596	577120	kdkdk-10-eeeee	94	93	GTGAAGCGAAGTGCACACGG	224
1577	1592	577127	kkk-8-eeeee	95	70	AGCGAAGTGCACACGG	1334
1577	1592	577134	kek-8-eeeee	94	89	AGCGAAGTGCACACGG	1334
1577	1592	577135	kek-10-kek	96	94	AGCGAAGTGCACACGG	1334
1583	1598	552859*	ekk-10-kke	92	91	AGGTGAAGCGAAGTGC	1340
1583	1602	577121	kdkdk-10-eeeee	91	74	GCAGAGGTGAAGCGAAGTGC	226
1583	1598	577128	kkk-8-eeeee	92	85	AGGTGAAGCGAAGTGC	1340
1583	1598	577132	kdkdk-9-ee	97	81	AGGTGAAGCGAAGTGC	1340
1583	1598	577136	kek-10-kek	95	95	AGGTGAAGCGAAGTGC	1340
1588	1603	566832	kdkdk-9-ee	95	78	TGCAGAGGTGAAGCGA	1345
1780	1795	552870*	ekk-10-kke	71	93	TTTATGCCTACAGCCT	232
1780	1799	577122	kdkdk-10-eeeee	70	96	CCAATTTATGCCTACAGCCT	50
1780	1796	577125	kdkdk-8-eeee	70	94	ATTTATGCCTACAGCCT	51
1780	1795	577129	kkk-8-eeeee	76	51	TTTATGCCTACAGCCT	232
1780	1795	577133	kdkdk-9-ee	80	52	TTTATGCCTACAGCCT	232
1873	1892	9591*	Uniform deoxy	30	14	CACCCAAGGCACAGCTTGG	1377

Example 19: Efficacy of gapmers targeting HBV in transgenic mice

Transgenic mice were treated with ISIS antisense oligonucleotides in a number of studies to evaluate the efficacy of the gapmers. HBV DNA and RNA levels were assessed.

Study 1

Groups of 12 mice each were injected subcutaneously twice a week for 4 weeks with 50 mg/kg of ISIS 510106, ISIS 510116, ISIS 505347, or ISIS 509934. A control group of 12 mice was injected subcutaneously twice a week for 4 weeks with PBS. Mice were euthanized 48 hours after the last dose, and livers were harvested for further analysis.

DNA and RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of HBV DNA, using primer probe sets RTS3370, RTS3371, and RTS3372. The DNA levels were normalized to picogreen. HBV RNA samples were also assayed with primer probe sets RTS3370 and RTS3371 after RT-PCR analysis. The mRNA levels were normalized to RIBOGREEN®. The data is presented in Table 56, expressed as percent inhibition compared to the control group. As shown in Table 56, most of the antisense oligonucleotides achieved reduction of HBV DNA and RNA over the PBS control. Results are presented as percent inhibition of HBV mRNA or DNA, relative to control. The Chemistry column indicates the gap-wing motif of each gapmer.

Table 56

Percent inhibition of HBV RNA and DNA in the liver of transgenic mice

ISIS No	Chemistry	% inhibition DNA (RTS3370)	% inhibition DNA (RTS3371)	% inhibition DNA (RTS3372)	% inhibition RNA (RTS3370)	% inhibition RNA (RTS3371)	% inhibition RNA (RTS3372)
505347	5-10-5 MOE	72	79	75	54	28	30
509934	5-10-5 MOE	93	95	94	72	75	92
510106	3-10-4 MOE	0	0	51	0	0	12
510116	3-10-4 MOE	68	79	68	49	54	66

Study 2

Groups of 6 mice each were injected subcutaneously twice a week for 4 weeks with 50 mg/kg of ISIS 146779, ISIS 505358, ISIS 146786, ISIS 509974, ISIS 509958, or ISIS 509959. A control group of 10 mice was injected subcutaneously twice a week for 4 weeks with PBS. Mice were euthanized 48 hours after the last dose, and livers were harvested for further analysis.

DNA and RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of HBV DNA, using primer probe sets RTS3370. The DNA levels were normalized to picogreen. HBV RNA samples were also assayed with primer probe sets RTS3370 after RT-PCR analysis. The mRNA levels were normalized to RIBOGREEN®. The data is presented in Table 57, expressed as percent inhibition compared to the control group. As shown in Table 57, most of the antisense oligonucleotides achieved reduction of HBV DNA and RNA over the PBS control. Results are presented as percent inhibition of HBV mRNA or DNA, relative to control. The Chemistry column indicates the gap-wing motif of each gapmer.

Table 57

Percent inhibition of HBV RNA and DNA in the liver of transgenic mice

ISIS No	Chemistry	% inhibition DNA	% inhibition RNA
146779	5-10-5 MOE	39	5
146786	5-10-5 MOE	83	73
505358	5-10-5 MOE	84	77
509958	3-10-3 MOE	82	29
509959	3-10-3 MOE	54	30
509974	3-10-3 MOE	56	28

Study 3

Groups of 6 mice each were injected subcutaneously twice a week for 4 weeks with 50 mg/kg of ISIS 509960, ISIS 505329, ISIS 146786, ISIS 505339, or ISIS 509927. Another group of 6 mice was administered Entecavir, an oral antiviral drug used to treate Hepatitis B infection, at 1 mg/kg daily for two weeks. A control group of 10 mice was injected subcutaneously twice a week for 4 weeks with PBS. Mice were euthanized 48 hours after the last dose, and livers were harvested for further analysis.

DNA and RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of HBV DNA, using primer probe sets RTS3371. The DNA levels were normalized to picogreen. HBV RNA samples were also assayed with primer probe sets RTS3371 after RT-PCR analysis. The mRNA levels were normalized to RIBOGREEN®. The data is presented in Table 58, expressed as percent inhibition compared to the control group. As shown in Table 58, most of the antisense oligonucleotides achieved reduction of HBV DNA and RNA over the PBS control. Results are presented as percent inhibition of HBV mRNA or DNA, relative to control. The Chemistry column indicates the gap-wing motif of each gapmer.

Table 58

Percent inhibition of HBV RNA and DNA in the liver of transgenic mice

	Oligo Chemistry	% inhibition DNA	% inhibition RNA
entecavir	-	94	0
ISIS 146786	5-10-5 MOE	97	92
ISIS 505329	5-10-5 MOE	70	63
ISIS 505339	5-10-5 MOE	74	63
ISIS 509927	5-10-5 MOE	80	57
ISIS 509960	3-10-3 MOE	86	60

Study 4

Groups of 6 mice each were injected subcutaneously twice a week for 4 weeks with 25 mg/kg of ISIS 146786, ISIS 552176, and ISIS 552073. One group of 10 mice was injected subcutaneously twice a week for 4 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

DNA and RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of HBV DNA, using primer probe set RTS3371. The DNA levels were normalized to picogreen. HBV RNA samples were also assayed with primer probe set RTS3371 after RT-PCR analysis. The mRNA levels were normalized to RIBOGREEN®. The data is presented in Table 59. As shown in Table 59, the antisense oligonucleotides achieved reduction of HBV DNA and RNA over the PBS control. Results are presented as percent inhibition of HBV mRNA or DNA, relative to control. The Chemistry column indicates the gap-wing motif of each gapmer.

Table 59

Percent inhibition of HBV RNA and DNA in transgenic mice

ISIS No	Chemistry	% inhibition of RNA	% inhibition of DNA
146786	5-10-5 MOE	81	91
552073	8-10-2 MOE	39	22
552176	3-9-5 MOE	55	56

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of ALT were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY)

(Nyblom, H. et al., Alcohol & Alcoholism 39: 336-339, 2004; Tietz NW (Ed): Clinical Guide to Laboratory Tests, 3rd ed. W. B. Saunders, Philadelphia, PA, 1995). The results are presented in Table 60 expressed in IU/L. Both the ISIS oligonucleotides were considered tolerable in the mice, as demonstrated by their liver transaminase profile.

Table 60

ALT levels (IU/L) of transgenic mice

	ALT
PBS	77
ISIS 146786	21
ISIS 552073	19
ISIS 552176	27

Study 5

Groups of 6 mice each were injected subcutaneously twice a week for 4 weeks with 25 mg/kg of ISIS 146786, ISIS 552056, ISIS 552088, and ISIS 552309. One group of 10 mice was injected subcutaneously twice a week for 4 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

DNA and RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of HBV DNA, using primer probe set RTS3371. The DNA levels were normalized to picogreen. HBV RNA samples were also assayed with primer probe set RTS3371 after RT-PCR analysis. The mRNA levels were normalized to RIBOGREEN®. As shown in Table 61, the antisense oligonucleotides achieved reduction of HBV DNA and RNA over the PBS control. Results are presented as percent inhibition of HBV mRNA or DNA, relative to control. The Chemistry column indicates the gap-wing motif of each gapmer.

Table 61

Percent inhibition of HBV DNA and RNA in transgenic mice

	Chemistry	% inhibition (RNA)	% inhibition (DNA)
ISIS 146786	5-10-5 MOE	60	90
ISIS 552056	7-10-3 MOE	25	58
ISIS 552088	8-10-2 MOE	8	0
ISIS 552309	5-9-3 MOE	35	84

Study 6

Groups of 6 mice each were injected subcutaneously twice a week for 4 weeks with 25 mg/kg of ISIS 146786, ISIS 505330, ISIS 509932, ISIS 552032, ISIS 552057, ISIS 552075, ISIS 552092, and ISIS 552255. One group of 10 mice was injected subcutaneously twice a week for 4 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

DNA and RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of HBV DNA, using primer probe set RTS3371. The DNA levels were normalized to picogreen. HBV RNA samples were also assayed with primer probe set RTS3371 after RT-PCR analysis. The mRNA levels were normalized to RIBOGREEN®. As shown in Table 62, the antisense oligonucleotides achieved reduction of HBV DNA and RNA over the PBS control. Results are presented as percent inhibition of HBV mRNA or DNA, relative to control. The Chemistry column indicates the gap-wing motif of each gapmer.

Table 62

Percent inhibition of HBV DNA and RNA in transgenic mice

ISIS No	Chemistry	% inhibition (RNA)	% inhibition (DNA)
146786	5-10-5 MOE	52	95
505330	5-10-5 MOE	7	61
509932	5-10-5 MOE	83	98
552032	6-10-4 MOE	54	97
552057	7-10-3 MOE	19	62
552075	8-10-2 MOE	12	18
552092	8-10-2 MOE	25	74
552255	4-9-4 MOE	41	89

Study 7

Groups of 6 mice each were injected subcutaneously twice a week for 4 weeks with 20 mg/kg of ISIS 552859, ISIS 577121, ISIS 577122, ISIS 577123, ISIS 577132, ISIS 577133, and ISIS 577134. These gapmers have deoxy, MOE and (S)-cEt chemistry. One group of 10 mice was injected subcutaneously twice a week for 4 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

DNA and RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of HBV DNA, using primer probe set RTS3371. The DNA levels were normalized to picogreen. HBV RNA samples were also assayed with primer probe set RTS3371 after RT-PCR analysis. The mRNA levels were normalized to RIBOGREEN®. As shown in Table 63, the antisense oligonucleotides achieved reduction of HBV DNA and RNA over the PBS control. Results are presented as percent inhibition of HBV mRNA or DNA, relative to control. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; the number indicates the number of deoxynucleosides; otherwise, 'd' indicates a deoxynucleoside; and 'e' indicates a MOE modification.

Table 63

Percent inhibition of HBV DNA and RNA in transgenic mice

ISIS No	Chemistry	% inhibition (RNA)	% inhibition (DNA)
552859	ekk-10-kke	60	86
577121	kdkdk-10-eeeee	59	93
577122	kdkdk-10-eeeee	42	68
577123	eekk-9-ekee	0	77
577132	kdkdk-9-ee	4	24
577133	kdkdk-9-ee	46	64
577134	kek-8-eeeee	0	17

Study 8

A group of 6 mice was injected subcutaneously twice a week for 4 weeks with 25 mg/kg of ISIS 146786, the 5-10-5 MOE gapmer. Groups of 6 mice each were injected subcutaneously twice a week for 4 weeks with 10 mg/kg of ISIS 552803, ISIS 552903, ISIS 552817, ISIS 552822, and ISIS 552907. These gapmers all had deoxy, MOE, and (S)-cEt chemistry. One group of 10 mice was injected subcutaneously twice a week for 4 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

DNA and RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of HBV DNA, using primer probe set RTS3371. The DNA levels were normalized to picogreen. HBV RNA samples were also assayed with primer probe set RTS3371 after RT-PCR analysis. The mRNA levels were normalized to RIBOGREEN®. The data is presented in Table 64. As shown in Table 64, the antisense oligonucleotides achieved reduction of HBV DNA and RNA over the PBS control. The 'Chemistry' column describes the sugar modifications of

each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; the number indicates the number of deoxynucleosides; otherwise, 'd' indicates a deoxynucleoside; and 'e' indicates a MOE modification; in case of the MOE gapmers, the Chemistry column defines the gap-wing structure.

Table 64

Percent inhibition of HBV RNA and DNA in transgenic mice

ISIS No	Chemistry	Dose (mg/kg/wk)	% inhibition of RNA	% inhibition of DNA
146786	5-10-5 MOE	50	81	91
552803	ekk-10-kke	20	71	95
552817	ekk-10-kke	20	86	51
552822	ekk-10-kke	20	90	89
552903	ek-10-keke	20	56	82
552907	ek-10-keke	20	41	45

Study 9

A group of 6 mice was injected subcutaneously twice a week for 4 weeks with 25 mg/kg of ISIS 146786. Groups of 6 mice each were injected subcutaneously twice a week for 4 weeks with 10 mg/kg of ISIS 552853, ISIS 552854, ISIS 552932, and ISIS 552938. One group of 10 mice was injected subcutaneously twice a week for 4 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

DNA and RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of HBV DNA, using primer probe set RTS3371. The DNA levels were normalized to picogreen. HBV RNA samples were also assayed with primer probe set RTS3371 after RT-PCR analysis. The mRNA levels were normalized to RIBOGREEN®. As shown in Table 65, the antisense oligonucleotides achieved reduction of HBV DNA and RNA over the PBS control. Results are presented as percent inhibition of HBV mRNA or DNA, relative to control. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; the number indicates the number of deoxynucleosides; otherwise, 'd' indicates a deoxynucleoside; and 'e' indicates a MOE modification; in case of the MOE gapmers, the Chemistry column defines the gap-wing structure.

Table 65

Percent inhibition of HBV DNA and RNA in transgenic mice

	Chemistry	Dose (mg/kg/wk)	% inhibition (DNA)	% inhibition (RNA)
ISIS 146786	5-10-5 MOE	50	90	60
ISIS 552853	ekk-10-kke	20	94	60
ISIS 552854	ekk-10-kke	20	61	23
ISIS 552932	ek-10-keke	20	75	70
ISIS 552938	ek-10-keke	20	67	56

Study 10

A group of 6 mice was injected subcutaneously twice a week for 4 weeks with 25 mg/kg of ISIS 146786. Groups of 6 mice each were injected subcutaneously twice a week for 4 weeks with 10 mg/kg of ISIS 552922, ISIS 552923, ISIS 552942, ISIS 552872, ISIS 552925, ISIS 552937, and ISIS 552939. One group of 10 mice was injected subcutaneously twice a week for 4 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

DNA and RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of HBV DNA, using primer probe set RTS3371. The DNA levels were normalized to picogreen. HBV RNA samples were also assayed with primer probe set RTS3371 after RT-PCR analysis. The mRNA levels were normalized to RIBOGREEN®. As shown in Table 66, the antisense oligonucleotides achieved reduction of HBV DNA and RNA over the PBS control. Results are presented as percent inhibition of HBV mRNA or DNA, relative to control. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; the number indicates the number of deoxynucleosides; otherwise, 'd' indicates a deoxynucleoside; and 'e' indicates a MOE modification; in case of the MOE gapmers, the Chemistry column defines the gap-wing structure.

Table 66

Percent inhibition of HBV DNA and RNA in transgenic mice

ISIS No	Chemistry	Dose (mg/kg/wk)	% inhibition (DNA)	% inhibition (RNA)
146786	5-10-5 MOE	50	52	57
552922	ekk-10-kke	20	61	50
552923	ek-10-keke	20	89	76
552942	ek-10-keke	20	58	52

552872	ek-10-keke	20	77	46
552925	ek-10-keke	20	89	65
552937	ek-10-keke	20	59	35
552939	ek-10-keke	20	57	19

Example 20: Efficacy of gapmers targeting HBV in transgenic mice

Mice harboring a HBV gene fragment (Guidotti, L. G. et al., *J. Virol.* **1995**, *69*, 6158-6169) were used. The mice were treated with ISIS antisense oligonucleotides selected from studies described above and evaluated for their efficacy in this model. HBV DNA, RNA, and antigen levels were assessed.

Groups of 10 mice each were injected subcutaneously twice a week for the first with 50 mg/kg and, subsequently, twice a week for the next 3 weeks with 25 mg/kg of ISIS 146786 or ISIS 510100. Control groups of 10 mice each were treated in a similar manner with ISIS 141923 (CCTTCCCTGAAGGTTCCTCC, SEQ ID NO: 320; 5-10-5 MOE gapmer with no known murine target) or ISIS 459024 (CGGTCCTTGGAGGATGC, SEQ ID NO: 1351; 3-10-4 MOE gapmer with no known murine target). Mice were euthanized 48 hours after the last dose, and organs and serum were harvested for further analysis.

DNA and RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of HBV DNA, using primer probe sets RTS3370, RTS3371, or RTS3372 (forward sequence ATCCTATCAACACTTCCGGAAACT, designated SEQ ID NO: 314; reverse sequence CGACGCGGCGATTGAG, designated SEQ ID NO: 315; probe sequence AAGAACTCCCTCGCCTCGCAGACG, designated SEQ ID NO: 316). The DNA levels were normalized to picogreen. HBV RNA samples were also assayed with primer probe sets RTS3370 and RTS3371 after RT-PCR analysis. The mRNA levels were normalized to RIBOGREEN®. The data is presented in Table 67. Serum DNA samples were analyzed after the study period. The data is presented in Table 68, expressed relative to the levels measured in the control group. As shown in Tables 67 and 68, the antisense oligonucleotides achieved reduction of HBV DNA and RNA over the PBS control. Results are presented as percent inhibition of HBV mRNA or DNA, relative to control. The Chemistry column defines the gap-wing structure of each gapmer.

Table 67

Percent inhibition of HBV RNA and DNA in the liver of transgenic mice

ISIS No	Chemistry	% inhibition DNA (RTS3370)	% inhibition DNA (RTS3371)	% inhibition DNA (RTS3372)	% inhibition RNA (RTS3370)	% inhibition RNA (RTS3371)	% inhibition RNA (RTS3372)
146786	5-10-5 MOE	97	97	95	86	85	89
510100	3-10-4 MOE	95	94	94	56	64	77

141923	5-10-5 MOE	2	0	13	0	7	31
459024	3-10-4 MOE	19	0	8	0	0	0

Table 68

Percent inhibition of HBV DNA in the serum of transgenic mice

ISIS No	% inhibition (RTS3370)	% inhibition (RTS3371)
146786	98	98
510100	99	98
141923	0	0
459024	0	0

HBV antigen Analysis

HBV antigens in the supernatants were detected with the ELISA technique. HBs antigen (HBsAg) levels were detected by ELISA from Abazyme LLC, MA. As presented in Table 57, treatment with ISIS oligonucleotides 146786 or 510100 caused reduction in HBsAg levels. HBe antigen (HBeAg) levels were detected by ELISA from International Immuno-diagnostics, CA. As presented in Table 69, treatment with ISIS oligonucleotides 146786 or 510100 also caused reduction in HBeAg levels.

Table 69

HBV antigen levels (PEI U/mL) in transgenic mice

	HBsAg	HBeAg
PBS	40	80
146786	3	15
510100	15	22
141923	32	80
459024	44	51

Example 21: Antisense inhibition of HBV viral mRNA in HepG2 cells by deoxy, MOE and (S)-cEt gapmers

Additional antisense oligonucleotides were designed targeting a HBV viral nucleic acid and were tested for their effects on HBV mRNA in vitro. ISIS 146786, ISIS 505358, ISIS 509932, and ISIS 510100, disclosed in U.S. Provisional Application No. 61/478,040 filed on April 21, 2011; ISIS 552859 disclosed in U.S. Provisional Application No. 61/596692 filed on February 8, 2012; ISIS 577121, ISIS 577122, ISIS 577123, ISIS 577132, ISIS 577133, and ISIS 577134, disclosed in the study described above, were also

included in the assay. Cultured HepG2 cells at a density of 28,000 cells per well were transfected using Cytofectin with 9.375 nM, 18.75 nM, 37.50 nM, 75.00 nM, 150.00 nM, or 300.00 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe set RTS3371 was used to measure mRNA levels. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of HBV, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in Tables below were designed as deoxy, MOE and (S)-cEt gapmers. The deoxy, MOE and (S)-cEt gapmers are 16, 17, or 18 nucleosides in length wherein the nucleosides have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; the number indicates the number of deoxynucleosides; otherwise, 'd' indicates a deoxynucleoside; and 'e' indicates a MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each oligonucleotide are 5-methylcytosines.

"Viral Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the viral gene sequence. "Viral Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted viral gene sequence. Each gapmer listed in Table 70 is targeted to the viral genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. U95551.1).

Table 70

Chimeric antisense oligonucleotides targeting SEQ ID NO: 1

Viral Target Start Site	Viral Target Stop Site	ISIS No	Motif	Sequence	SEQ ID NO
411	427	585163	eeekk-8-eeee	GGCATAGCAGCAGGATG	17
414	430	585164	eeekk-7-kkeee	TGAGGCATAGCAGCAGG	21
414	430	585165	eeek-9-keee	TGAGGCATAGCAGCAGG	21
1577	1593	585170	eeekk-7-kkeee	AAGCGAAGTGCACACGG	1304
1577	1593	585171	eeek-9-keee	AAGCGAAGTGCACACGG	1304
1577	1593	585172	eeeekk-7-eeee	AAGCGAAGTGCACACGG	1304
1577	1593	585173	ekek-9-eeee	AAGCGAAGTGCACACGG	1304
1577	1593	585174	ekekdk-7-eeee	AAGCGAAGTGCACACGG	1304
1583	1599	585166	eeekk-7-kkeee	GAGGTGAAGCGAAGTGC	1310
1583	1599	585167	eeek-9-keee	GAGGTGAAGCGAAGTGC	1310
1780	1797	577119	kdkdk-8-eeeee	AATTTATGCCTACAGCCT	1379
1780	1796	585168	eeekk-7-kkeee	ATTTATGCCTACAGCCT	51
1780	1796	585169	eeek-9-keee	ATTTATGCCTACAGCCT	51

Table 71

Dose dependent inhibition of HBV mRNA levels by chimeric antisense oligonucleotides

ISIS No	9.375 nM	18.75 nM	37.5 nM	75.0 nM	150.0 nM	300.0 nM
146786	37	37	58	70	81	93
505358	30	26	28	57	74	85
510100	42	30	43	61	77	91
552859	21	30	39	61	79	91
577119	42	43	46	66	74	75
577121	10	15	42	64	82	89
577122	21	30	53	66	78	84
577123	27	29	45	56	78	84
577132	14	21	42	61	80	92
577133	12	14	32	47	62	77
577134	37	39	59	72	86	90
585174	31	28	48	61	80	90

Table 72

Dose dependent inhibition of HBV mRNA levels by chimeric antisense oligonucleotides

ISIS No	9.375 nM	18.75 nM	37.5 nM	75.0 nM	150.0 nM	300.0 nM
146786	25	34	57	71	85	92
509932	9	28	59	62	70	74
585163	17	32	52	68	77	81
585164	23	4	29	31	36	56
585165	6	31	42	58	66	82
585166	19	27	35	48	50	63
585167	22	25	50	69	76	88
585168	4	30	44	52	67	76
585169	32	32	42	62	76	80
585170	23	19	39	49	66	75
585171	28	27	42	59	81	88
585172	26	29	30	64	80	91
585173	29	30	41	71	86	88

Example 22: Analysis of the potency of uniform deoxyoligonucleotides in inhibition of HBV mRNA in HepG2 cells

Additional antisense oligonucleotides were tested for their effects on HBV mRNA in vitro. ISIS 5808 and ISIS 9591, disclosed in US5985662 were also included in the assay. ISIS 146786 was included in

the assay as the benchmark. Cultured HepG2 cells at a density of 28,000 cells per well were transfected using LipofectAMINE2000® with 18.75 nM, 37.50 nM, 75.00 nM, 150.00 nM, or 300.00 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and HBV mRNA levels were measured by quantitative real-time PCR. Viral primer probe set RTS3371 was used to measure mRNA and DNA levels. HBV mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. S antigen and E antigen levels were also measured by ELISA. Results are presented as percent inhibition, relative to untreated control cells.

The antisense oligonucleotides tested, ISIS 582699, ISIS 582700, and ISIS 582701, were designed according to the sequences and chemistries disclosed in *Korba and Gerin, Antiviral Research, 1995, Vol. 28, 225-242*; the corresponding names for the oligonucleotides in the reference are S1, C1, and L2c, respectively. The antisense oligonucleotides in Tables below were designed as uniform deoxy oligonucleotides, 16 or 21 nucleosides in length wherein the nucleosides have deoxy modifications. "Viral Target start site" indicates the 5'-most nucleotide to which the oligonucleotide is targeted in the viral gene sequence. "Viral Target stop site" indicates the 3'-most nucleotide to which the oligonucleotide is targeted viral gene sequence. Each oligonucleotide listed in Table 73 is targeted to the viral genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. U95551.1). The results indicate that the deoxy oligonucleotides had negligible effect on HBV mRNA expression levels DNA levels and HBV antigen levels.

Table 73
Uniform deoxy oligonucleotides targeting SEQ ID NO: 1

Viral Target Start Site	Viral Target Stop Site	ISIS No	Sequence	SEQ ID NO
160	180	582699	GAATCCTGATGTGATGTTCTC	1378
1884	1899	582701	CCAAAGCCACCCAAGG	1380
1910	1930	582700	CAAATTCTTTATAAGGGTCGA	1381

Table 74
Dose dependent inhibition of HBV mRNA levels after treatment with oligonucleotides

ISIS No	18.75 nM	37.5 nM	75.0 nM	150.0 nM	300.0 nM
5808	38	23	29	40	54
9591	35	20	32	26	40
146786	11	5	45	66	92
582699	32	28	27	39	52
582700	18	12	20	16	23
582701	4	0	0	3	13

Table 75

Dose dependent inhibition of HBV DNA levels in HepG2 cells after treatment with oligonucleotides

ISIS No	18.75 nM	37.5 nM	75.0 nM	150.0 nM
5808	20	17	0	0
9591	0	0	0	0
146786	32	50	77	83
582699	0	44	0	17
582700	0	0	0	0
582701	0	0	0	0

Table 76

HBV S antigen levels after treatment with oligonucleotides (arbitrary units)

ISIS No	18.75 nM	37.5 nM	75.0 nM	150.0 nM
5808	9,254	8,228	4,168	2,540
9591	10,924	8,683	9,334	12,142
146786	12,501	7,265	3,408	1,017
582699	9,340	9,325	7,589	4,712
582700	9,697	8,350	11,168	10,703
582701	15,283	18,209	14,632	15,299

5

Table 77

HBV E antigen levels after treatment with oligonucleotides (arbitrary units)

ISIS No	18.75 nM	37.5 nM	75.0 nM	150.0 nM
5808	8,075	8,587	5,036	3,286
9591	9,242	8,093	8,257	6,944
146786	8,532	4,034	2,301	449
582699	7,815	7,191	7,026	5,278
582700	8,690	9,304	7,941	6,315
582701	8,847	8,257	8,211	6,276

CLAIMS

What is claimed is:

1. A compound, comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 5-310, 321-802, 804-1272, or 1288-1350, 1364-1372, 1375, 1376, and 1379.

2. A compound, comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides, wherein said modified oligonucleotide is complementary to SEQ ID NO: 1

3. The compound of claim 1 or 2, wherein said modified oligonucleotide is at least 96% complementary to SEQ ID NO: 1.

4. The compound of claim 1 or 2, wherein said modified oligonucleotide is at least 97% complementary to SEQ ID NO: 1.

5. The compound of claim 1 or 2, wherein said modified oligonucleotide is at least 98% complementary to SEQ ID NO: 1.

6. The compound of claim 1 or 2, wherein said modified oligonucleotide is at least 99% complementary to SEQ ID NO: 1.

7. The compound of claim 1 or 2, wherein said modified oligonucleotide is 100% complementary to SEQ ID NO: 1.

8. The compound of claim 1 or 2, consisting of a single-stranded modified oligonucleotide.

9. The compound of claim 1 or 2, wherein at least one internucleoside linkage is a modified internucleoside linkage.

10. The compound of claim 9, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.

11. The compound of claim 1 or 2, wherein at least one nucleoside of the modified oligonucleotide comprises a modified sugar.

12. The compound of claim 11, wherein the at least one modified sugar is a bicyclic sugar.

13. The compound of claim 11, wherein at least one modified sugar comprises a 2'-O-methoxyethyl group.

14. The compound of claim 11, wherein the modified sugar comprises a 2'-$O(CH_2)_2$-OCH_3 group.

15. The compound of claim 11, wherein the modified sugar comprises a 4'-$CH(CH_3)$-O-2' group.

16. The compound of claim 1 or 2, wherein at least one nucleoside comprises a modified nucleobase.

17. The compound of claim 16, wherein the modified nucleobase is a 5-methylcytosine.

18. The compound of claim 1 or 2, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

19. The compound of claim 18, wherein the gap segment consists of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 linked nucleosides.

20. The compound of claim 18, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of 2-3 linked nucleosides; and

a 3' wing segment consisting of 3-4 linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar or a constrained ethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage.

21. The compound of claim 20, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of 3 linked nucleosides; and

a 3' wing segment consisting of 3 linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar or a constrained ethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage.

22. The compound of claim 21, wherein each of the 3 linked nucleosides of the 5' wing segment comprises a 2'-O-methoxyethyl sugar and each of the 3 linked nucleosides of the 3' wing segment comprises a constrained ethyl sugar.

23. The compound of claim 21, wherein the 3 linked nucleosides of the 5' wing segment comprise a 2'-O-methoxyethyl sugar, a constrained ethyl sugar, and a constrained ethyl sugar in the 5' to 3' direction, and the 3 linked nucleosides of the 3' wing segment comprise a constrained ethyl sugar, a constrained ethyl sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction.

24. The compound of claim 20, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of 2 linked nucleosides; and

a 3' wing segment consisting of 4 linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar or a constrained ethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage.

25. The compound of claim 24, wherein the 2 linked nucleosides of the 5' wing segment comprise a 2'-O-methoxyethyl sugar and a constrained ethyl sugar in the 5' to 3' direction, and the 4 linked nucleosides of

the 3' wing segment comprise a constrained ethyl sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction.

26. The compound of claim 18, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of five linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

27. The compound of claim 18, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of four linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

28. The compound of claim 18, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

29. The compound of claim 18, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of two linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

30. The compound of claim 18, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

31. The compound of claim 18, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of 1-5 linked nucleosides; and

a 3' wing segment consisting of 1-5 linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage.

32. The compound of claim 1 or 2, wherein the modified oligonucleotide consists of 20 linked nucleosides.

33. The compound of claim 1 or 2, wherein the modified oligonucleotide consists of 16 linked nucleosides.

34. The compound of claim 1 or 2, wherein said modified oligonucleotide consists of 15 to 30 linked nucleosides.

35. The compound of claim 1 or 2, wherein said modified oligonucleotide consists of 18 to 22 linked nucleosides.

36. The compound of claim 1 or 2, wherein said modified oligonucleotide consists of 20 to 30 linked nucleosides.

37. A compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides complementary within nucleotides 1-20, 10-29, 10-56, 13-38, 13-35, 19-38, 25-47, 25-50, 25-56, 43-68, 43-63, 55-74, 58-73, 58-74, 58-77, 58-79, 58-80, 58-84, 59-74, 59-75, 59-80, 60-75, 60-76, 60-79, 61-76, 61-77, 61-80, 62-77, 63-84, 68-114, 101-123, 98-123, 113-138, 116-138, 131-150, 137-162, 152-186, 158-177, 167-186, 191-215, 196-224, 196-215, 196-218, 199-228, 199-218, 199-224, 200-224, 205-224, 206-228, 218-237, 224-243, 233-264, 242-263, 243-262, 244-263, 245-274; 245-260, 245-264, 246-266, 247-266, 247-269, 247-270, 245-267, 251-267, 245-266, 250-269, 251-266, 251-268, 251-269, 245-269, 245-266, 245-261, 250-265, 250-266, 250-267, 250-268, 250-269, 251-270, 252-267, 253-268, 253-269, 253-272, 253-274, 254-269, 254-270, 254-274, 255-270, 255-271, 255-274, 255-401, 255-400, 255-274, 255-273, 255-272, 255-271, 256-271, 256-275, 255-276, 256-272, 256-276, 253-275, 256-279, 257-276, 258-273, 259-274, 260-279, 262-281, 262-321, 262-315, 262-312, 265-312, 266-288, 266-291, 266-285, 281-321, 281-303, 290-321, 290-312, 292-311, 290-312, 293-312, 293-315, 293-321, 296-321, 302-321, 324-343, 339-361, 339-367, 348-367, 342-367, 358-392, 358-378, 360-392, 360-383, 360-388, 360-385, 362-381, 366-388, 369-388, 366-385, 366-392, 370-389, 370-392, 380-399, 382-401, 384-433, 384-400, 384-401, 385-401, 405-424, 409-428, 405-428, 411-426, 411-427, 411-430, 411-431, 411-437, 412-431, 411-426, 411-427, 412-428, 412-431, 412-427, 413-433, 413-432, 413-428, 413-429, 413-432, 413-433, 414-427, 415-427, 414-429, 414-430, 414-433, 415-428, 415-429, 415-430, 415-431, 415-434, 416-431, 416-432, 416-429, 416-435, 417-432, 417-433, 417-436, 418-433, 418-435, 418-434, 418-437, 419-435, 419-434, 420-435, 419-432, 419-434, 421-436, 422-437, 422-441, 423-436, 425-465, 454-473, 454-472, 457-476, 457-472, 457-473, 454-476, 455-472, 457-485, 458-485, 458-483, 458-477, 458-473, 459-485, 460-485, 463-498, 463-485, 466-485, 463-482, 457-491, 458-491, 459-491, 460-491, 463-491, 466-491, 472-491, 472-493, 473-492, 475-491, 459-494, 460-494, 463-494, 466-494, 467-498, 472-494, 475-494, 457-473, 457-472, 458-494, 454-494, 457-494, 457-473, 485-513, 470-493, 476-519, 485-519, 500-519, 512-534, 512-550, 524-546, 536-559, 548-567, 548-570, 550-570, 548-594, 554-573, 548-576, 560-594, 584-606, 611-645, 617-363, 623-642, 617-645, 639-754, 639-658, 639-654, 641-656, 642-657, 643-658, 642-754, 653-672, 662-685, 665-685,665-689, 668-687, 670-754, 670-706, 670-685, 670-686, 670-689, 671-690, 671-691, 671-686, 671-687, 672-693, 672-697, 672-707, 672-687, 672-688, 673-688, 674-693, 678-693, 679-694, 679-707, 679-698, 679-701, 679-702, 679-707, 680-695, 680-699, 679-699, 681-706, 681-696, 682-697, 682-706, 682-707, 682-702, 682-701, 683-698, 684-699, 685-700, 686-701, 687-754, 688-704, 689-709, 689-710, 690-705, 679-705, 679-710, 679-706, 690-710, 691-710, 690-754, 690-706, 684-703, 687-705, 687-702, 687-703, 687-706, 688-703, 688-704, 688-705, 689-704, 689-705, 689-708, 690-705, 690-706, 690-709, 691-706, 692-711, 693-716, 693-712, 695-715, 697-716, 697-716, 690-716, 724-746, 724-752, 724-754, 724-758, 733-752, 738-754, 738-753, 739-758, 739-754, 739-775, 739-754, 740-754, 742-785, 742-773, 757-776, 757-785, 790-815, 793-812, 811-833, 811-844, 814-833, 811-906,

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1976, 2035-2057, 2083-2141, 2230-2261, 2368-2393, 2381-2397, 2368-2394, 2379-2394, 2381-2396, 2368-2397, 2368-2396, 2420-2439, 2458-2476, 2459-2478, 2819-2838, 2818-2838, 2873-2892, or 3161-3182 of SEQ ID NO: 1, wherein said modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

38. A compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases 100% complementary to an equal length portion of nucleobases 1-20, 10-29, 10-56, 13-38, 13-35, 19-38, 25-47, 25-50, 25-56, 43-68, 43-63, 55-74, 58-73, 58-74, 58-77, 58-79, 58-80, 58-84, 59-74, 59-75, 59-80, 60-75, 60-76, 60-79, 61-76, 61-77, 61-80, 62-77, 63-84, 68-114, 101-123, 98-123, 113-138, 116-138, 131-150, 137-162, 152-186, 158-177, 167-186, 191-215, 196-224, 196-215, 196-218, 199-228, 199-218, 199-224, 200-224, 205-224, 206-228, 218-237, 224-243, 233-264, 242-263, 243-262, 244-263, 245-274; 245-260, 245-264, 246-266, 247-266, 247-269, 247-270, 245-267, 251-267, 245-266, 250-269, 251-266, 251-268, 251-269, 245-269, 245-266, 245-261, 250-265, 250-266, 250-267, 250-268, 250-269, 251-270, 252-267, 253-268, 253-269, 253-272, 253-274, 254-269, 254-270, 254-274, 255-270, 255-271, 255-274, 255-401, 255-400, 255-274, 255-273, 255-272, 255-271, 256-271, 256-275, 255-276, 256-272, 256-276, 253-275, 256-279, 257-276, 258-273, 259-274, 260-279, 262-281, 262-321, 262-315, 262-312, 265-312, 266-288, 266-291, 266-285, 281-321, 281-303, 290-321, 290-312, 292-311, 290-312, 293-312, 293-315, 293-321, 296-321, 302-321, 324-343, 339-361, 339-367, 348-367, 342-367, 358-392, 358-378, 360-392, 360-383, 360-388, 360-385, 362-381, 366-388, 369-388, 366-385, 366-392, 370-389, 370-392, 380-399, 382-401, 384-433, 384-400, 384-401, 385-401, 405-424, 409-428, 405-428, 411-426, 411-427, 411-430, 411-431, 411-437, 412-431, 411-426, 411-427, 412-428, 412-431, 412-427, 413-433, 413-432, 413-428, 413-429, 413-432, 413-433, 414-427, 415-427, 414-429, 414-430, 414-433, 415-428, 415-429, 415-430, 415-431, 415-434, 416-431, 416-432, 416-429, 416-435, 417-432, 417-433, 417-436, 418-433, 418-435, 418-434, 418-437, 419-435, 419-434, 420-435, 419-432, 419-434, 421-436, 422-437, 422-441, 423-436, 425-465, 454-473, 454-472, 457-476, 457-472, 457-473, 454-476, 455-472, 457-485, 458-485, 458-483, 458-477, 458-473, 459-485, 460-485, 463-498, 463-485, 466-485, 463-482, 457-491, 458-491, 459-491, 460-491, 463-491, 466-491, 472-491, 472-493, 473-492, 475-491, 459-494, 460-494, 463-494, 466-494, 467-498, 472-494, 475-494, 457-473, 457-472, 458-494, 454-494, 457-494, 457-473, 485-513, 470-493, 476-519, 485-519, 500-519, 512-534, 512-550, 524-546, 536-559, 548-567, 548-570, 550-570, 548-594, 554-573, 548-576, 560-594, 584-606, 611-645, 617-363, 623-642, 617-645, 639-754, 639-658, 639-654, 641-656, 642-657, 643-658, 642-754, 653-672, 662-685, 665-685,665-689, 668-687, 670-754, 670-706, 670-685, 670-686, 670-689, 671-690, 671-691, 671-686, 671-687, 672-693, 672-697, 672-707, 672-687, 672-688, 673-688, 674-693, 678-693, 679-694, 679-707, 679-698, 679-701, 679-702, 679-707, 680-695, 680-699, 679-699, 681-706, 681-696, 682-697, 682-706, 682-707, 682-702, 682-701, 683-698, 684-699, 685-700, 686-701,

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1815-1844, 1815-1840, 1815-1834, 1818-1837, 1821-1840, 1821-1844, 1821-1837, 1822-1843, 1822-1839, 1822-1837, 1823-1843, 1823-1838, 1824-1839, 1827-1846, 1861-1884, 1861-1880, 1865-1885, 1866-1881, 1867-1882, 1867-1886, 1868-1883, 1869-1885, 1869-1884, 1870-1885, 1871-1886, 1872-1887, 1874-1889, 1876-1895, 1888-1914, 1888-1908, 1891-1910, 1891-1914, 1895-1938, 1895-1935, 1913-1935, 1898-1920, 1907-1929, 1913-1935, 1918-1934, 1919-1938, 1919-1934, 1921-1934, 1928-1956, 1957-1976, 2035-2057, 2083-2141, 2230-2261, 2368-2393, 2381-2397, 2368-2394, 2379-2394, 2381-2396, 2368-2397, 2368-2396, 2420-2439, 2458-2476, 2459-2478, 2819-2838, 2818-2838, 2873-2892, or 3161-3182, wherein the nucleobase sequence of the modified oligonucleotide is complementary to SEQ ID NO: 1.

39. The compound of claim 36, wherein at least one nucleoside of the modified oligonucleotide comprises at least one 2'-O-methoxyethyl sugar or constrained ethyl sugar.

40. A compound, comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 1, 1273, 1274, 1275, 1276, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1284, 1285, 1287, 1352, 1353, 1354, 1359, 1360, 1361, 1362, and 1363.

41. A composition comprising the compound of any of claims 1-40 or salt thereof and at least one of a pharmaceutically acceptable carrier or diluent.

42. A method comprising administering to an animal the compound or composition of any of claims 1-41.

43. The method of claim 42, wherein the animal is a human.

44. The method of claim 43, wherein administering the compound prevents, treats, ameliorates, or slows progression of a HBV-related disease, disorder or condition.

45. The method of claim 44, wherein the disease, disorder or condition is liver disease.

46. The method of claim 44, wherein the disease, disorder or condition is jaundice, liver inflammation, liver fibrosis, inflammation, liver cirrhosis, liver failure, diffuse hepatocellular inflammatory disease, hemophagocytic syndrome, serum hepatitis, HBV viremia, or liver disease–related transplantation.

47. The method of claim 44, wherein the disease or condition is a hyperproliferative condition.

48. The method of claim 47, wherein the hyperproliferative condition is liver cancer.

49. A method of reducing antigen levels in an animal comprising administering to said animal the compound or composition of any of claims 1-41.

50. The method of claim 49, wherein HBsAG levels are reduced.

51. The method of claim 49, wherein HBeAG levels are reduced.

52. The method of claim 49, wherein the animal is human.

53. The method of claim 49, comprising co-administering the compound or composition and a second agent.

54. The method of claim 53, wherein the compound or composition and the second agent are administered concomitantly.