

MODULATION OF HEPATITIS B VIRUS (HBV) EXPRESSION

RELATED APPLICATIONS

[0001] This application claims the priority and benefit of U.S. Provisional Application No. 63/298,092, filed on January 10, 2022, the contents of which are incorporated herein in its entirety.

BACKGROUND

[0002] 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>).

[0003] 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.

[0004] 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 (INF α), 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 IFN α 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.

[0005] 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.

[0006] 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. 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.

[0007] HBV antisense oligonucleotides which possess a single gap segment flanked directly between a 5' wing segment, and a 3' wing segment have been developed (WO 2012/145697). However, many of the HBV antisense oligonucleotides with this structure exhibited minimal efficacy in reducing serum HBsAg level and/or caused safety concerns in Chronic Hepatitis B patients. Thus there exists a need in the art for improved HBV antisense oligonucleotides.

SUMMARY

[0008] The present disclosure extends the principles of HBV antisense oligonucleotides by providing enhanced modified oligonucleotide structures that provide further segmented gap structures, with the use of one or more Separators.

[0009] The present disclosure is based, at least in part, on the finding that a discontinuous gap segment flanked between a 5' wing segment and a 3' wing segment in a modified oligonucleotide provides improved activity over conventional anti-sense oligonucleotides that have a continuous gap (e.g., reduction of serum HBsAg levels or HBeAg levels). One or more separator segments placed directly in between gap segments can provide improved activity of the anti-sense oligonucleotide, which is distinct from the current teachings in the art, which show that separator segments can lead to poor activity. The inventors have unexpectedly discovered that the placement of separator segments at specific positions of the full length anti-sense oligonucleotide provides improved activity, while separator segments in other positions results in reduced activity or no changes to activity. Further, only specific types of nucleoside modifications in the separator segments provide improved activity, while other types of nucleoside modifications in the separator segments results in reduced activity or no changes to activity. Without being bound by theory, the specific combination of nucleoside modification and positioning of the separator segment within the full length anti-sense oligonucleotide, improves binding and activity of RNase H endonuclease, without compromising the complementarity to the HBV target sequence.

[0010] The present disclosure is based, at least in part, on the finding that specific nucleoside modifications of the 5' wing segment and 3' wing segment decrease *in vivo* toxicity (e.g., reduction in ALT levels, a proxy for liver toxicity, or CC30 (cytotoxic concentration to reduce cell viability by 30%)). The inventors have unexpectedly discovered specific types of modified nucleosides in the 5' wing segment causes increased *in vivo* toxicity, whereas specific types of modified nucleosides in the 3' wing segment decreased *in vivo* toxicity. Further, the positioning of the modified nucleosides within the 5' wing segment and 3' wing segment also contribute to *in vivo* toxicity. Without being bound by theory, the specific combination of nucleoside modification and positioning within the 5' wing segment and the 3' wing segment, contributes to the complementarity of the full length anti-sense oligonucleotide to the HBV target sequence. While the combination of specific types of modifications of at certain positions improves complementarity, other combinations of specific types of modifications and positions lowers complementarity, causing increased off-target binding, which ultimately leads to toxicity.

[0011] 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.

[0012] 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.

[0013] 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).

[0014] 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.

[0015] 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIG. 1 provides a legend for the nucleoside modifications at each position of the sequences shown in FIG. 2. The column “Examples” describes each type of nucleoside modification.

[0017] FIG. 2 shows a table of exemplary modified oligonucleotides of the disclosure. The modifications at each position of the modified oligonucleotide sequences are read using the legend in FIG. 1. AUS1010 to AUS1714 (SEQ ID NO: 11 to SEQ ID NO: 666) represent the modified oligonucleotide sequences. AUS1233 (SEQ ID NO: 10) , also referred to as AUS1138, is a reference modified oligonucleotide sequence.

[0018] FIG. 3 shows a schematic diagram for the solid-phase synthesis process used to produce the modified oligonucleotides.

[0019] FIG. 4 shows a schematic diagram of a modified oligonucleotide dosing regimen and HBsAg level sampling schedule for HBV Tg mice.

[0020] FIG. 5 shows a schematic diagram of a modified oligonucleotide dosing regimen and HBsAg level sampling schedule for C57BL/6 Tg mice.

[0021] FIG. 6 shows a schematic diagram of a modified oligonucleotide dosing regimen and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels sampling schedule for C57BL/6 Tg mice.

[0022] FIG. 7 shows a schematic diagram of a modified oligonucleotide dosing regimen and HBsAg level sampling schedule for pAAV-1.2HBV-GTA HDI-HBV mice.

[0023] FIG. 8 shows a schematic diagram of a modified oligonucleotide dosing regimen and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels sampling schedule for C57BL/6 Tg mice.

[0024] FIG. 9 shows a schematic diagram of an exemplary modified oligonucleotide dosing regimen and HBsAg level sampling schedule for pcDNA3.1-preS2-GTD HDI-HBV mice.

[0025] FIG. 10 shows a schematic diagram of an exemplary modified oligonucleotide dosing regimen and HBsAg level sampling schedule for pcDNA3.1-preS2-GTA HDI-HBV mice.

[0026] FIG. 11 shows a schematic diagram of an exemplary modified oligonucleotide dosing regimen and HBsAg level sampling schedule for GTA HBV Tg mice.

[0027] FIG. 12 shows a schematic diagram of an exemplary modified oligonucleotide dosing regimen and HBsAg level sampling schedule for GTD AAV-HBV Tg mice.

[0028] FIG. 13 shows a schematic diagram of an exemplary modified oligonucleotide dosing regimen and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels sampling schedule for C57BL/6 Tg mice.

[0029] FIG. 14 represents Table 15.1, which shows the serum levels of HBsAg at days 3, 7, 10, 14, 21, and 28 in GT-C 1.0HBV transgenic mice following a 40mg/kg subcutaneous treatment of the modified oligonucleotides AUS1233, AUS1683, AUS1684, AUS1685, AUS1220, AUS1322, AUS1323, or AUS1324. The mean values are expressed in $\log_{10}$.

[0030] FIG. 15 represents Table 15.2, which shows the serum levels of HBsAg at days 3, 7, 10, 14, 21, and 28 in GT-C 1.0HBV transgenic mice following a 40mg/kg subcutaneous treatment of the modified oligonucleotides AUS1169, AUS1171, AUS1170, AUS1168, or AUS1322. The mean values are expressed in $\log_{10}$.

[0031] FIG. 16 represents Table 15.3, which shows the serum levels of HBsAg at days 3, 7, 10, 14, and 21 in GT-C 1.0HBV transgenic mice following a 30mg/kg subcutaneous treatment of the modified oligonucleotides AUS1434, AUS1435, AUS1440, AUS1436, AUS1437, AUS1438, AUS1439, AUS1441, AUS1422, AUS1423, AUS1424, AUS1425, AUS1426, AUS1427, AUS1428, AUS1429, AUS1430, AUS1431, AUS1432, or AUS1433. The mean values are expressed in $\log_{10}$.

[0032] FIG. 17 represents Table 15.4, which shows the serum levels of HBsAg at days 3, 7, 10, 14, and 21 in GT-C 1.0HBV transgenic mice following a 30mg/kg subcutaneous treatment of the modified oligonucleotides AUS1463, AUS1466, AUS1476, AUS1472, AUS1489, or AUS1459. The mean values are expressed in $\log_{10}$.

[0033] FIG. 18 represents Table 15.5, which shows the serum levels of HBsAg at days 3, 7, 10, 14, 21, and 28 in GT-C 1.0HBV transgenic mice following a 30mg/kg subcutaneous treatment of the modified oligonucleotides AUS1492, AUS1495, AUS1494, AUS1493, AUS1482, or AUS1441. The mean values are expressed in $\log_{10}$.

[0034] FIG. 19 represents Table 15.6, which shows the serum levels of HBsAg at days 3, 7, 10, 14, and 21 in GT-C 1.0HBV transgenic mice following a 40mg/kg subcutaneous treatment of the

modified oligonucleotides AUS1693, AUS1694, AUS1695, AUS1696, AUS1697, AUS1698, AUS1699, or AUS1700. The mean values are expressed in $\log_{10}$.

[0035] FIG. 20 represents Table 15.7, which shows the serum levels of HBsAg at days 3, 7, 10, 14, 21, and 28 in GT-C 1.0HBV transgenic mice following treatment with modified oligonucleotides. Rows 1 and 2 show HBsAg levels following a 10mg/kg subcutaneous dose of AUS1493 or AUS1233 on Day 0. Rows 3 and 4 show HBsAg levels following a 30mg/kg subcutaneous dose of AUS1493 or AUS1233 on Day 0. The mean values are expressed in $\log_{10}$.

[0036] FIG. 21 represents Table 16.1, which shows the serum levels of HBsAg at days 3, 7, 10, 14, 21, and 28 in GT-D HDI HBV mice following a 60mg/kg subcutaneous treatment of the eASO compound AUS1683, AUS1684, AUS1685, AUS1220, AUS1322, AUS1323, or AUS1324. The mean values are expressed in $\log_{10}$.

[0037] FIG. 22 represents Table 16.2, which shows the serum levels of HBsAg at days 3, 7, 10, 14, 21, and 28 in GT-D HDI HBV mice following a 60mg/kg subcutaneous treatment of the eASO compound AUS1169, AUS1171, AUS1170, or AUS1168. The mean values are expressed in $\log_{10}$.

[0038] FIG. 23 represents Table 16.3, which shows the serum levels of HBsAg at days 3, 7, 10, 14, and 21 in GT-D HDI HBV mice following a 60mg/kg subcutaneous treatment of the eASO compound AUS1173, AUS1174, AUS1175, or AUS1176. The mean values are expressed in $\log_{10}$.

[0039] FIG. 24 represents Table 16.4, which shows the serum levels of HBsAg at days 3, 7, 10, and 14 in GT-D HDI HBV mice following a 60mg/kg subcutaneous treatment of the eASO compound AUS1173, AUS1177, AUS1178, AUS1179, AUS1180, AUS1181, AUS1182, AUS1194, AUS1184, AUS1185, AUS1186, AUS1187, AUS1188, AUS1189, AUS1190, AUS1191, AUS1192, AUS1193, AUS1183, AUS1239, AUS1325, AUS1326, AUS1327, AUS1328, AUS1329, AUS1175, AUS1361, or AUS1362. The mean values are expressed in $\log_{10}$.

[0040] FIG. 25 represents Table 16.5, which shows the serum levels of HBsAg at days 3, 7, 10, 14, and 22 in GT-D HDI HBV mice following a 60mg/kg subcutaneous treatment of the eASO compound AUS1322, AUS1382, AUS1383, AUS1384, or AUS1385. The mean values are expressed in $\log_{10}$.

[0041] FIG. 26 represents Table 16.6, which shows the serum levels of HBsAg at days 3, 7, 10, 14, and 21 in GT-D HDI HBV mice following a 60mg/kg subcutaneous treatment of the eASO

compound AUS1322, AUS1388, AUS1389, AUS1390, AUS1392, AUS1393, AUS1396, AUS1403, AUS1409, AUS1411, AUS1413, AUS1414, or AUS1415. The mean values are expressed in $\log_{10}$.

[0042] FIG. 27 represents Table 16.7, which shows the serum levels of HBsAg at days 3, 7, 10, and 14 in GT-D HDI HBV mice following a 60mg/kg subcutaneous treatment of the eASO compound AUS1387, AUS1326, AUS1328, AUS1388, AUS1390, AUS1403, or AUS1360. The mean values are expressed in $\log_{10}$.

[0043] FIG. 28 represents Table 16.8, which shows the serum levels of HBsAg at days 3, 7, 10, 14, and 21 in GT-D HDI HBV mice following a 40mg/kg subcutaneous treatment of the eASO compound AUS1434, AUS1435, AUS1440, AUS1436, AUS1437, AUS1438, AUS1439, AUS1441, AUS1422, AUS1423, AUS1424, AUS1425, AUS1426, AUS1427, AUS1428, AUS1429, AUS1430, AUS1431, AUS1432, or AUS1433. The mean values are expressed in $\log_{10}$.

[0044] FIG. 29 represents Table 16.9, which shows the serum levels of HBsAg at days 3, 7, 10, 14, and 21 in GT-D HDI HBV (1.5ug/ml pcDNA3.1 preS2/S plasmid) mice following a 40mg/kg subcutaneous treatment of the eASO compound AUS1444, AUS1458, AUS1459, AUS1460, AUS1461, AUS1462, AUS1463, AUS1464, AUS1465, AUS1466, AUS1467, AUS1468, AUS1469, AUS1470, AUS1471, AUS1472, AUS1473, AUS1474, AUS1475, AUS1476, AUS1477, AUS1478, AUS1479, AUS1480, AUS1481, AUS1482, AUS1483, AUS1488, AUS1489, AUS1490, AUS1443, AUS1444, or AUS1445. The mean values are expressed in $\log_{10}$.

[0045] FIG. 30 represents Table 16.10, which shows the serum levels of HBsAg at days 3, 7, 10, 14, 21, and 28 in GT-D HDI HBV (1.5ug/ml pcDNA3.1 preS2/S plasmid) mice following a 15mg/kg or 45mg/kg subcutaneous treatment of the eASO compound AUS1492, AUS1493, AUS1233, AUS1492, or AUS1493. The mean values are expressed in $\log_{10}$.

[0046] FIG. 31 represents Table 16.11, which shows the serum levels of HBsAg at days 3, 7, 10, 14, 21, and 28 in GT-A HDI HBV (1.5ug/ml pcDNA3.1 preS2/S plasmid) mice following a 40mg/kg subcutaneous treatment of the eASO compound AUS1492, AUS1493, AUS1233, AUS1492, or AUS1493. The mean values are expressed in $\log_{10}$.

[0047] FIG. 32 represents Table 16.12, which shows the serum levels of HBsAg at days 3, 7, 10, 14, and 21 in GT-A HDI HBV (1.5ug/ml pcDNA3.1 preS2/S plasmid) mice following a 15

or 45 mg/kg subcutaneous treatment of the eASO compound AUS1441, AUS1466, AUS1472, AUS1488, or AUS1489. The mean values are expressed in $\log_{10}$.

[0048] FIG. 33 represents Table 16.13, which shows the serum levels of HBsAg at days 3, 7, 10, 14, 21, and 28 in GT-D HDI HBV (1.5ug/ml pcDNA3.1 preS2/S plasmid) mice following a 15 or 45 mg/kg subcutaneous treatment of the eASO compound AUS1495, AUS1494, AUS1233, AUS1495, AUS1494, AUS1492, or AUS1441. The mean values are expressed in $\log_{10}$.

[0049] FIG. 34 represents Table 16.14, which shows the serum levels of HBsAg at days 3, 7, 10, 14, 21, and 28 in GT-A HDI HBV (1.5ug/ml pcDNA3.1 preS2/S plasmid) mice following a 15 or 45 mg/kg subcutaneous treatment of the eASO compound AUS1495, AUS1494, AUS1233, AUS1495, AUS1494, AUS1492, AUS1482, or AUS1441. The mean values are expressed in $\log_{10}$.

[0050] FIG. 35 represents Table 16.15, which shows the serum levels of HBsAg at days 3, 7, 10, 14, and 21 in GT-B HDI HBV (0.5ug/ml pcDNA3.1 preS2/S plasmid) mice following a 40mg/kg subcutaneous treatment of the eASO compound AUS1684. The mean values are expressed in $\log_{10}$.

[0051] FIG. 36 represents Table 16.16, which shows the serum levels of HBsAg at days 3, 7, 10, 14, 21, and 28 in GT-A 1.2HBV mice following a 45mg/kg subcutaneous treatment of the eASO compound AUS1492 or AUS1493. The mean values are expressed in $\log_{10}$.

[0052] FIG. 37 represents Table 16.17, which shows the serum levels of HBsAg at days 3, 7, 10, 14, and 21 in GT-A HDI HBV (1.5ug/ml pAAV-1.2HBV plasmid) mice following a 40mg/kg subcutaneous treatment of the eASO compound AUS1396, AUS1422, AUS1423, AUS1424, AUS1425, AUS1426, AUS1427, AUS1428, AUS1429, AUS1430, AUS1431, AUS1432, or AUS1433. The mean values are expressed in $\log_{10}$.

[0053] FIG. 38 represents Table 16.18, which shows the serum levels of HBsAg at days 3, 7, 10, 14, and 21 in GT-A HDI HBV (1.5ug/ml pAAV-1.2HBV plasmid) mice following a 40mg/kg subcutaneous treatment of the eASO compound AUS1444, AUS1458, AUS1459, AUS1460, AUS1461, AUS1462, AUS1463, AUS1464, AUS1465, AUS1466, AUS1467, AUS1468, AUS1469, AUS1470, AUS1471, AUS1472, AUS1473, AUS1474, AUS1475, AUS1476, AUS1477, AUS1478, AUS1479, AUS1480, AUS1481, AUS1482, AUS1483, AUS1488, AUS1489, AUS1490, AUS1443, or AUS1434. The mean values are expressed in $\log_{10}$.

[0054] FIG. 39 represents Table 17, which shows the serum levels of HBsAg, HBeAg, and HBV DNA at days 3, 7, 10, 14, 21, and 28 in GT-D AAV HBV (rAAV-HBV1.3-mer WT replicon) mice following a 40mg/kg subcutaneous treatment of the eASO compound AUS1493. The mean values are expressed in log₁₀.

[0055] FIG. 40 represents Table 18.1 which shows the serum levels of ALT in WT male C57BL/6 mice at days 5, 7, 10, 14, 17, and 21 following a 60mg/kg subcutaneous treatment of the eASO compound AUS1434, AUS1435, AUS1440, AUS1436, AUS1437, AUS1438, AUS1439, AUS1441, AUS1178, AUS1190, AUS1188, AUS1192, AUS1422, AUS1423, AUS1424, AUS1425, AUS1426, AUS1427, AUS1428, AUS1429, AUS1430, AUS1431, AUS1432, AUS1433, AUS1360, AUS1401, AUS1361, AUS1362, or AUS1411 on days 0, 2, and 4.

[0056] FIG. 41 represents Table 18.2 which shows the serum levels of ALT in WT male C57BL/6 mice at days 5, 7, 10, 14, 17, and 21 following a 60mg/kg subcutaneous treatment of the eASO compound AUS1443, AUS1444, AUS1445, AUS1446, AUS1447, AUS1448, AUS1449, AUS1450, AUS1452, AUS1453, AUS1454, AUS1455, AUS1456, or AUS1457 on days 0, 2, and 4.

DETAILED DESCRIPTION

[0057] 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.

[0058] 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

[0059] 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.

[0060] Unless otherwise indicated, the following terms have the following meanings:

[0061] “2’-O-methoxyethyl” (also 2’-MOE and 2’-O(CH₂)₂-OCH₃) refers to an O-methoxyethyl modification at the 2’ position of a furanose ring. A 2’-O-methoxyethyl modified sugar is a modified sugar.

[0062] “2’-MOE nucleoside” (also 2’-O-methoxyethyl nucleoside) means a nucleoside comprising a 2’-MOE modified sugar moiety.

[0063] “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.

[0064] “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.

[0065] “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.

[0066] “5-methylcytosine” means a cytosine modified with a methyl group attached to the 5 position. A 5-methylcytosine is a modified nucleobase.

[0067] “About” means within $\pm 7\%$ 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%.

[0068] “Acceptable safety profile” means a pattern of side effects that is within clinically acceptable limits.

[0069] “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.

[0070] “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.

[0071] “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.

[0072] “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.

[0073] “Administering” means providing a pharmaceutical agent to an individual, and includes, but is not limited to administering by a medical professional and self-administering.

[0074] “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.

[0075] “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.

[0076] “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.

[0077] “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.

[0078] “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.

[0079] “Antisense compound” means an oligomeric compound that 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.

[0080] “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.

[0081] “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.

[0082] “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.

[0083] The “area under the curve” or “AUC” is the integral of the concentration of a drug in blood plasma as a function of time. The AUC can be determined for the totality of time for which data is available, for example until the drug is no longer detectable (AUC_{0-t}), the area under the curve from time 0 extrapolated to infinity (AUC_{0-∞}), or for a particular truncated window of time, for example 24 hours after administration (AUC₀₋₂₄).

[0084] “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.

[0085] “Bicyclic sugar” means a furanose ring modified by the bridging of two non-geminal carbon atoms. A bicyclic sugar is a modified sugar.

[0086] “Body weight” refers to an animal’s whole body weight, inclusive of all tissues including adipose tissue.

[0087] “Cap structure” or “terminal cap moiety” means chemical modifications, which have been incorporated at either terminus of an antisense compound.

[0088] “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(CH₃)-O-2’.

[0089] “Constrained ethyl nucleoside” (also cEt nucleoside) means a nucleoside comprising a bicyclic sugar moiety comprising a 4’-CH(CH₃)-O-2’ bridge.

[0090] “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.

[0091] “Chimeric antisense compounds” means antisense compounds that have at least 2 chemically distinct regions, each position having a plurality of subunits.

[0092] “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.

[0093] “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.

[0094] “Complementarity” means the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

[0095] “Comply” means the adherence with a recommended therapy by an individual.

[0096] “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.

[0097] “Contiguous nucleobases” means nucleobases immediately adjacent to each other.

[0098] “Cure” means a method or course that restores health or a prescribed treatment for an illness.

[0099] “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.

[0100] “Designing” or “Designed to” refer to the process of designing an oligomeric compound that specifically hybridizes with a selected nucleic acid molecule.

[0101] “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.

[0102] “Dosage unit” means a form in which a pharmaceutical agent is provided, e.g. pill, tablet, or other dosage unit known in the art.

[0103] “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.

[0104] "Dosing regimen" is a combination of doses designed to achieve one or more desired effects.

[0105] "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.

[0106] "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.

[0107] "Efficacy" means the ability to produce a desired effect.

[0108] "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.

[0109] The term "fragment," as applied to a polynucleotide, will be understood to mean a nucleotide sequence of reduced length relative to a reference nucleic acid or nucleotide sequence and comprising, consisting essentially of, and/or consisting of a nucleotide sequence of contiguous nucleotides identical or almost identical (e.g., 60%, 70%, 80%, 90%, 92%, 95%, 98% or 99% identical) to the reference nucleic acid or nucleotide sequence. Such a nucleic acid fragment according to the invention may be, where appropriate, included in a larger polynucleotide of which it is a constituent. In some embodiments, such fragments can comprise, consist essentially of, and/or consist of oligonucleotides having a length of at least about 8, 10, 12, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, or more consecutive nucleotides of a nucleic acid or nucleotide sequence according to the invention.

[0110] "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.

[0111] "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.

[0112] “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 can be chemically distinct from the nucleoside or nucleosides comprising the external regions. The internal region may be referred to as the “gap” and each of the 5’ and 3’ external regions may be referred to as a “wing.”

[0113] A “gap” is an internal segment of a chimeric antisense compound that comprises one or more linked deoxynucleosides, and is positioned between a 5’ wing (W1) and a 3’ wing (W2). A gap may be referred to as a “gap” a “gap region” or a “gap segment”.

[0114] “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.

[0115] “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”.

[0116] “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).

[0117] “HBV mRNA” means any messenger RNA expressed by hepatitis B virus.

[0118] “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.

[0119] “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 “HBsAg” and envelope proteins such as “HBV surface antigen”, or “HBsAg”.

[0120] “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 gg/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_{1/2}, 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-IFN α 2a therapy (Moucari et al (2009) Hepatology 49:1151).

[0121] “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.

[0122] “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.

[0123] As used herein, the term “IC₅₀” or “IC₅₀ value” refers to the concentration of an agent where cell viability is reduced by half. The IC₅₀ is thus a measure of the effectiveness of an agent in inhibiting a biological process.

[0124] “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.

[0125] “Immediately adjacent” means there are no intervening elements between the immediately adjacent elements.

[0126] “Individual” means a human or non-human animal selected for treatment or therapy.

[0127] “Individual compliance” means adherence to a recommended or prescribed therapy by an individual.

[0128] “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. The term “inhibit” or “reduce” or grammatical variations thereof, as used herein, refer to a decrease or diminishment in the specified level or activity of at least about 5%, about 10%, about 15%, about 25%, about 35%, about 40%, about 50%, about 60%, about 75%, about 80%, about 90%, about 95% or more. In some embodiments, the inhibition or reduction results in little or essentially no detectable activity (at most, an insignificant amount, *e.g.*, less than about 10% or even 5%).

[0129] “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.

[0130] “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.

[0131] “Injection site reaction” means inflammation or abnormal redness of skin at a site of injection in an individual.

[0132] “Internucleoside linkage” refers to the chemical bond between nucleosides.

[0133] “Intraperitoneal administration” means administration through infusion or injection into the peritoneum.

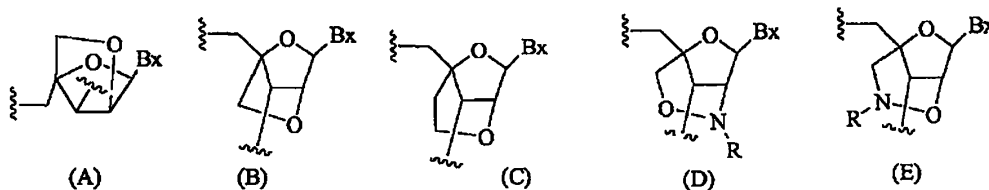
[0134] “Intravenous administration” means administration into a vein.

[0135] “Lengthened” antisense oligonucleotides are those that have one or more additional nucleosides relative to an antisense oligonucleotide disclosed herein.

[0136] “Linked deoxynucleoside” means a deoxyribonucleic acid base (A, G, C, T, U) linked by a phosphate ester to form a nucleotide.

[0137] “Linked nucleosides” means adjacent nucleosides linked together by an internucleoside linkage. An example of linked nucleoside is a linked nucleotide in which the linkage involves a phosphate group atom, *e.g.* a phosphodiester bond.

[0138] “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'-CH₂-O-2') LNA; (B) β -D-Methyleneoxy (4'-CH₂-O-2')-



LNA; (C) Ethyleneoxy (4'-(CH₂)₂-O-2') LNA; (D) Aminooxy (4'-CH₂-O-N(R)-2') LNA; and (E) Oxyamino (4'-CH₂-N(R)-O-2') LNA; as depicted below.

[0139] 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(R¹)(R²)]_n-, -C(R¹)=C(R²)-, -C(R¹)=N-, -C(=NR¹)-, -C(=O)-, -C(=S)-, -O-, -Si(R¹)₂-, -S(=O)_x- and -N(R¹)-, wherein: x is 0, 1, or 2; n is 1, 2, 3, or 4; each R¹ and R² is, independently, H, a protecting group, hydroxyl, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₅-C₂₀ aryl, substituted C₅-C₂₀ aryl, a heterocycle radical, a substituted heterocycle radical, heteroaryl, substituted heteroaryl, C₅-C₇ alicyclic radical, substituted C₅-C₇ alicyclic radical, halogen, OJ¹, NJ¹J², SJ¹, N₃, COOJ¹, acyl (C(=O)-H), substituted acyl, CN, sulfonyl (S(=O)₂-J¹), or sulfoxyl (S(=O)-J¹); and each J¹ and J² is, independently, H, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₅-C₂₀ aryl, substituted C₅-C₂₀ aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C₁-C₁₂ aminoalkyl, substituted C₁-C₁₂ aminoalkyl or a protecting group.

[0140] Examples of 4'-2' bridging groups encompassed within the definition of LNA include, but are not limited to one of formulae: --[C(R¹)(R²)]_n-, -[C(R¹)(R²)]_n-O-, -C(R¹)(R²)-N(R¹)-O- or -C(R¹)(R²)-O-N(R¹)-. Furthermore, other bridging groups encompassed with the definition of LNA are 4'-CH₂-2', 4'-(CH₂)₂-2', 4'-(CH₂)₃-2', 4'-CH₂-O-2', 4'-(CH₂)₂-O-2', 4'-CH₂-O-N(R¹)-2' and 4'-CH₂-N(R¹)-O-2'- bridges, wherein each R¹ and R² is, independently, H, a protecting group or C₁-C₁₂ alkyl.

[0141] 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'-CH₂-O-2') bridge to form the bicyclic sugar moiety. The bridge can also be a methylene (-CH₂-) group connecting the 2' oxygen atom and the 4' carbon atom, for which the term methyleneoxy (4'-CH₂-O-2') LNA is used. Furthermore, in the case of the bicyclic sugar moiety having an ethylene bridging group in this position, the term ethyleneoxy (4'-CH₂CH₂-O-2') LNA is used. α -L-methyleneoxy (4'-CH₂-O-2'), an isomer of methyleneoxy (4'-CH₂-O-2') LNA is also encompassed within the definition of LNA, as used herein.

[0142] A "maximum blood plasma concentration" or "C_{max}" refers to the highest concentration of a drug in the blood plasma after dose of the drug is given to a subject. Methods of measuring the concentration of drugs will be known to persons of ordinary skill in the art, and include, *inter alia*, liquid chromatography, and tandem mass spectrometry.

[0143] "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.

[0144] "Modified internucleoside linkage" refers to a substitution or any change from a naturally occurring internucleoside bond (i.e. a phosphodiester internucleoside bond).

[0145] "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).

[0146] "Modified nucleoside" means a nucleoside having, independently, a modified sugar moiety and/or modified nucleobase.

[0147] "Modified nucleotide" means a nucleotide having, independently, a modified sugar moiety, modified internucleoside linkage, or modified nucleobase.

[0148] "Modified oligonucleotide" means an oligonucleotide comprising at least one modified internucleoside linkage, a modified sugar, and/or a modified nucleobase.

[0149] "Modified sugar" means substitution and/or any change from a natural sugar moiety.

[0150] "Monomer" refers to a single unit of an oligomer. Monomers include, but are not limited to, nucleosides and nucleotides, whether naturally occurring or modified.

[0151] “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.

[0152] “Non-complementary nucleobase” refers to a pair of nucleobases that do not form hydrogen bonds with one another or otherwise support hybridization.

[0153] “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).

[0154] “Nucleobase” means a heterocyclic moiety capable of pairing with a base of another nucleic acid.

[0155] “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.

[0156] “Nucleobase sequence” means the order of contiguous nucleobases independent of any sugar, linkage, and/or nucleobase modification.

[0157] “Nucleoside” means a nucleobase linked to a sugar. A nucleoside includes deoxynucleosides, e.g. deoxyribonucleosides.

[0158] “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.

[0159] "Nucleotide" means a nucleoside having a phosphate group covalently linked to the sugar portion of the nucleoside.

[0160] "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.

[0161] "Oligomeric compound" means a polymer of linked monomeric subunits which is capable of hybridizing to at least a region of a nucleic acid molecule.

[0162] "Oligonucleoside" means an oligonucleotide in which the internucleoside linkages do not contain a phosphorus atom.

[0163] "Oligonucleotide" means a polymer of linked nucleosides each of which can be modified or unmodified, independent one from another.

[0164] "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.

[0165] "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.

[0166] "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.

[0167] "Pharmaceutically acceptable derivative" encompasses pharmaceutically acceptable salts, conjugates, prodrugs or isomers of the compounds described herein.

[0168] "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.

[0169] “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.

[0170] “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.

[0171] “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.

[0172] “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.

[0173] “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.

[0174] “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.

[0175] “Prophylactically effective amount” refers to an amount of a pharmaceutical agent that provides a prophylactic or preventative benefit to an animal.

[0176] “Recommended therapy” means a therapeutic regimen recommended by a medical professional for the treatment, amelioration, or prevention of a disease.

[0177] “Region” is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic.

[0178] “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.

[0179] “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.

[0180] “Segments” are defined as smaller or sub-portions of regions within a target nucleic acid.

[0181] A “separator” is a segment or region that separates two gap regions and is positioned in between two gap regions in a chimeric antisense compound. The separator segment may have one or more nucleosides, wherein the nucleosides are chemically distinct from the nucleosides comprising the gap. A separator segment comprises nucleosides modified to impart properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, lowered *in vivo* toxicity or resistance to degradation by *in vivo* nucleases. A chimeric antisense compound may comprise one or more separator segments. A separator may be referred to as a “separator” a “separator region” or a “separator segment”. Exemplary chimeric antisense compounds of the disclosure comprise one, two, three, four, five or six separator segments.

[0182] “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.

[0183] “Shortened” or “truncated” versions of antisense oligonucleotides taught herein have one, two or more nucleosides deleted.

[0184] “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.

[0185] “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.

[0186] “Sites,” as used herein, are defined as unique nucleobase positions within a target nucleic acid.

[0187] “Slows progression” means decrease in the development of the said disease.

[0188] “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.

[0189] “Statistically Significant,” as used herein means a measurable or observable parameter that is unlikely to occur by chance.

[0190] “Subcutaneous administration” means administration just below the skin. “Subject” means a human or non-human animal selected for treatment or therapy.

[0191] “Target” refers to a protein, the modulation of which is desired.

[0192] “Target gene” refers to a gene encoding a target.

[0193] “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.

[0194] “Target nucleic acid,” “target RNA,” “target RNA transcript” and “nucleic acid target” all mean a nucleic acid capable of being targeted by antisense compounds.

[0195] “Target region” means a portion of a target nucleic acid to which one or more antisense compounds is targeted.

[0196] “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.

[0197] “Therapeutically effective amount” means an amount of a pharmaceutical agent that provides a therapeutic benefit to an individual.

[0198] “Treatment” refers to administering a composition to effect an alteration or improvement of the disease or condition.

[0199] “Unmodified” nucleobases mean the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U).

[0200] “Unmodified nucleotide” means a nucleotide composed of naturally occurring nucleobases, sugar moieties, and internucleoside linkages. In certain embodiments, an unmodified nucleotide is an RNA nucleotide (i.e. (3-D-ribonucleosides) or a DNA nucleotide (i.e. (3-D-deoxyribonucleoside).

[0201] “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.

[0202] A “wing” is a terminal segment of a chimeric antisense oligonucleotide, modified to impart to an oligonucleotide, properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, lowered *in vivo* toxicity or resistance to degradation by *in vivo*

nucleases. A wing may be referred to as a “wing” a “wing region” or a “wing segment”. As used herein, a wing comprises at least two linked nucleosides; a subset of which may comprise one or more deoxynucleosides, but the entire wing cannot be made up of only deoxynucleosides.

[0203] The chimeric antisense compounds of the disclosure comprise a 5' wing segment (W1) located at the 5' terminus of the chimeric antisense oligonucleotide and the residue at the 3' end of the W1 cannot be a deoxynucleoside. The chimeric antisense compounds of the disclosure also comprise a 3' wing segment (W2) located at the 3' terminus of the chimeric antisense oligonucleotide and the 5' terminal residue of the W2 cannot be a deoxynucleoside.

[0204] A 5' wing (W1) begins at the 5' terminus of the chimeric antisense oligonucleotide, extends in the 5' to 3' direction, and ends at the first nucleoside that is not a deoxynucleoside, that is directly linked to a deoxynucleoside of a first gap, thus indicating the 3' end of the W1 and the 5' end of a first gap (G1).

[0205] A 3' wing (W2) begins at the 3' terminus of the chimeric antisense oligonucleotide, extends in the 3' to 5' direction, and ends at the first nucleoside that is not a deoxynucleoside, that is directly linked to a deoxynucleoside of a gap, thus indicating the 3' end of the last gap of and the 5' end of the W2.

Exemplary Chimeric Antisense Oligonucleotides

[0206] The disclosure provides for at least the following exemplary chimeric antisense oligonucleotides.

[0207] In one aspect, provide is an oligonucleotide that is modified, the modified oligonucleotide comprising from 5' to 3':

5' W1 - G1 - S1 - G2 - W2 3' (Formula I)

wherein:

W1 is a 5' wing segment;

W2 is a 3' wing segment;

G1 is a first gap segment;

S1 is a first separator segment;

G2 is a second gap segment;

- is an internucleoside linkage; and

at least one nucleoside of the oligonucleotide is modified.

[0208] In some embodiments, the modified oligonucleotide comprises from 5' to 3':

5' W1 - G1 - S1 - G2 - S2 - G3 - W2 3' (Formula II)

wherein:

S2 is a second separator segment; and

G3 is a third gap segment.

[0209] In some embodiments, the modified oligonucleotide comprises from 5' to 3':

5' W1 - G1 - S1 - G2 - S2 - G3 - S3 - G4 - W2 3' (Formula III)

wherein:

S3 is a third separator segment; and

G4 is a fourth gap segment.

[0210] In some embodiments, the modified oligonucleotide comprises from 5' to 3':

5' W1 - G1 - S1 - G2 - S2 - G3 - S3 - G4 - S4 - G5 - W2 3' (Formula IV)

wherein:

S4 is a fourth separator segment; and

G5 is a fifth gap segment.

[0211] In some embodiments, the modified oligonucleotide comprises from 5' to 3':

[0212] 5' W1 - G1 - S1 - G2 - S2 - G3 - S3 - G4 - S4 - G5 - S5 - G6 - W2 3' (Formula V)

wherein:

S5 is a fifth separator segment; and

G6 is a sixth gap segment.

[0213] In some embodiments, the modified oligonucleotide comprises from 5' to 3':

5' W1 - G1 - S1 - G2 - S2 - G3 - S3 - G4 - S4 - G5 - S5 - G6 - S6 - G7 - W2 3' (Formula VI)

wherein:

S6 is a sixth separator segment; and

G7 is a seventh gap segment.

[0214] In some embodiments, W1 comprises 2 to 25 linked nucleosides. In some embodiments, W1 comprises one or more linked deoxynucleosides.

[0215] In some embodiments, W2 comprises 2 to 35 linked nucleosides. In some embodiments, W2 comprises one or more linked deoxynucleosides.

[0216] In some embodiments, any one or more of G1, G2, G3, G4, G5, G6, and/or G7 comprises 1 to 10 linked deoxynucleosides.

[0217] In some embodiments, any one or more of S1, S2, S3, S4, S5, and/or S6 comprise 1, 2, 3, 4, or 5 linked nucleosides.

[0218] In some embodiments, the modified oligonucleotide is 18-50 nucleobases in length. In some embodiments, the modified oligonucleotide is at least 20 nucleobases in length. In some embodiments, the modified oligonucleotide is 20 nucleobases in length.

[0219] In some embodiments, the modified oligonucleotide is of Formula I, and W1 comprises 4-6 linked nucleosides, G1 comprises 1-6 linked deoxynucleosides, S1 comprises 1 linked nucleoside, G2 comprises 1-6 linked deoxynucleosides, and W2 comprises 4-6 linked nucleosides.

[0220] In some embodiments, the modified oligonucleotide is of Formula I, and W1 comprises 4-6 linked nucleosides, G1 comprises 5 linked deoxynucleosides, S1 comprises 1 linked nucleoside, G2 comprises 5 linked deoxynucleosides, and W2 comprises 4-6 linked nucleosides.

[0221] In some embodiments, the modified oligonucleotide is of Formula I, and W1 comprises 4 linked nucleosides, G1 comprises 5 linked deoxynucleosides, S1 comprises 1 linked nucleoside, G2 comprises 5 linked deoxynucleosides, and W2 comprises 5 linked nucleosides.

[0222] In some embodiments, the modified oligonucleotide is of Formula I, and the length of the G1-S1-G2 is between 8 and 12 nucleobases in length.

[0223] The following embodiments pertain to any of Formula I – VI: In some embodiments, G1, G2, G3, G4, G5, G6, and G7 can comprise a nucleoside comprising a modification to a 2'-deoxynucleoside. In some embodiments, any one or more of G1, G2, G3, G4, G5, G6, and G7 comprises a nucleoside comprising a 2'-deoxy 5-methylcytidine sugar modification. In some embodiments, S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-O-methoxyethyl sugar modification. In some embodiments, S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 5-methylcytidine. In some embodiments, S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-O-methyl sugar modification. In some embodiments, S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-OH sugar modification. In some embodiments, S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-fluoro sugar modification. In some embodiments, S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-fluoro-arabinonucleic acid (2'-fluoro-ANA) sugar modification. In some embodiments, S1, S2, S3, S4, S5, and/or S6 comprises a glycol nucleic acid (GNA). In some embodiments, S1, S2, S3, S4, S5, and/or S6 comprises a LNA. In some embodiments, W1 comprises a nucleoside comprising a 2'-deoxy sugar modification (e.g. wherein the 2'-deoxy sugar modification is at positions 2 and/or 5 of a sequence corresponding to SEQ ID NO: 2.). In some embodiments, W1 comprises a nucleoside comprising a 2'-O-methoxyethyl sugar modification (e.g. wherein the 2'-O-methoxyethyl sugar modification is at position 1, 2, 3, 4 and/or 5 of a

sequence corresponding to SEQ ID NO: 2). In some embodiments, W1 comprises a 2'-O-methoxyethyl 5-methylcytidine at position 2 of a sequence corresponding to SEQ ID NO: 2. In some embodiments, W1 comprises a nucleoside comprising a 2'-O-methyl sugar modification (e.g. wherein the 2'-O-methyl sugar modification is at positions 1, 2, 3, 4 and/or 5 of a sequence corresponding to SEQ ID NO: 2). In some embodiments, W1 comprises a 2'-O-methyl 5-methylcytidine at position 2 of a sequence corresponding to SEQ ID NO: 2. In some embodiments, W1 comprises a nucleoside comprising a 2'-fluoro sugar modification. In some embodiments, W1 comprises a nucleoside comprising a 2'-fluoro-arabinonucleic acid (2'-fluoro-ANA) modification. In some embodiments, W1 comprises a glycol nucleic acid (GNA) (e.g. wherein the GNA is at position 2 of a sequence corresponding to SEQ ID NO: 2.) In some embodiments, W1 comprises a modified nucleoside and wherein the modified nucleoside is a locked nucleic acid (LNA) (e.g. wherein the LNA is at positions 1 and/or 3 of a sequence corresponding to SEQ ID NO: 2). In some embodiments, W2 comprises a nucleoside comprising a 2'-deoxy sugar modification (e.g. wherein the 2'-deoxy sugar modification is at positions 15 and/or 16 of a sequence corresponding to SEQ ID NO: 2). In some embodiments, W2 comprises a nucleoside comprising a 2'-O-methoxyethyl sugar modification (e.g. wherein the 2'-O-methoxyethyl sugar modification is at positions 15, 16, 17, 18, 19 and/or 20 of a sequence corresponding to SEQ ID NO: 2). In some embodiments, W2 comprises a nucleoside comprising a 2'-O-methyl sugar modification (e.g. wherein the 2'-O-methyl sugar modification is at positions 15, 16, 17, 18, 19 and/or 20 of a sequence corresponding to SEQ ID NO: 2. In some embodiments, W2 comprises a nucleoside comprising a 2'-fluoro sugar modification. In some embodiments, W2 comprises a nucleoside comprising a 2'-fluoro-arabinonucleic acid (2'-fluoro-ANA) modification. In some embodiments, W2 comprises a modified nucleoside and wherein the modified nucleoside is a glycol nucleic acid (GNA). In some embodiments, W2 comprises a modified nucleoside and wherein the modified nucleoside is a locked nucleic acid (LNA) (e.g. wherein the LNA is at positions 16, 17, 18, 19 and/or 20 of a sequence corresponding to SEQ ID NO: 2.)

Exemplary Target Sequences and Exemplary Modified Oligonucleotides

[0224] Certain embodiments provide methods, compounds, and compositions for inhibiting HBV mRNA expression.

[0225] Certain embodiments provide antisense compounds targeted to a HBV nucleic acid sequence. Exemplary HBV nucleic acid sequences include but are not limited to those shown in Table 1.

Table 1 Exemplary HBV sequences

HBV Genotype	Accession No.	SEQ ID NO:
GTD (HepG2.2.15 cells)	U95551.1	SEQ ID NO: 3
GTA	AJ309369.1	SEQ ID NO: 667
GTB	D00330.1	SEQ ID NO: 668
GTC	AB033550.1	SEQ ID NO: 669
GTD	KX470733.1	SEQ ID NO: 670
GTE	KX186584	SEQ ID NO: 671
GTF	KP718112	SEQ ID NO: 672
GTG	KX264500	SEQ ID NO: 673
GTH	KX264501	SEQ ID NO: 674

[0226] In certain embodiments, the HBV nucleic acid is the sequence set forth in GENBANK Accession No. U95551.1 (incorporated herein as **SEQ ID NO: 3**). In some embodiments, the antisense compounds target a sequence recited in **SEQ ID NO: 3** or a portion thereof. In some embodiments, the antisense compounds target a sequence at positions 1583-1602 of **SEQ ID NO: 3**.

[0227] Exemplary HBV nucleic acid target sequences include but are not limited to those shown in Table 2.

Table 2. Exemplary HBV Target Sequences

HBV Target Sequence	SEQ ID NO:
CTTGG TCATG GGCCA TCAG	1
GCACT TCGCT TCACC TCTGC	4
TTTGTTTACGTCCCGTCGGC	675
TTTACGTCCCGTCGGCGCTGAATCCTGCGG	676
TTTACGTCCCGTCGGCGCTG	677
TTTACGCGGACTCCCCGTCT	678
TTGTTTACGTCCCGTCGGCG	679
TTGTTGACAAGAATCCTCAC	680
TTGAGAGAAGTCCACCACG	681
TTCTCCTGGCTCAGTTTACTAGTGCCATTT	682
TTCTCCTGGCTCAGTTTACT	683
TTACGTCCCGTCGGCGCTGA	684
TGTTTACGTCCCGTCGGCGC	685
TGTGCCTTCTCATCTGCCGG	686
TGTGCACTTCGCTTCACCTC	687
TGGTGGACTTCTCTCAATT	688
TGGCTCAGTTTACTAGTGCC	689
TGGACTTCTCTCAATTTTCTAGGGG	690

HBV Target Sequence	SEQ ID NO:
TGGACTTCTCTCAATTTTCT	691
TGCCTTCTCATCTGCCGGAC	692
TGCCGGACCGTGTGCACTTC	693
TGCCGATCCATACTGCCGAA	694
TGCACTTCGCTTCACCTCTG	695
TGACAAGAATCCTCACAATA	696
TCTTTACGCGGACTCCCCGT	697
TCTTGTTGACAAGAATCCTC	698
TCTGTGCCCTTCTCATCTGCC	699
TCTGCCGATCCATACTGCCG	700
TCTCTTTACGCGGACTCCCC	701
TCTCATCTGCCGGACCGTGT	702
TCGGCGCTGAATCCTGCCGA	703
TCCTGGCTCAGTTTACTAGT	704
TCCTCACAATACCGCAGAGT	705
TCCCGTCGGCGCTGAATCCT	706
TCCATACTGCCGGAACCTCTA	707
TCATCTGCCGGACCGTGTGC	708
TCATCTCGAACGTTACAGTCA	709
TCAGTTTACTAGTGCCATT	710
TATTGTGAGGATTCTTGTC	711
TAGGAGGCTGTAGGCATAAATTGGTCTGCG	712
TACGTCCCGTCGGCGCTGAA	713
TACGCGGACTCCCCGTCTGT	714
GTTTACGTCCCGTCGGCGCT	715
GTTGGTGCACTTCGCTTCAC	716
GTTGACAAGAATCCTCACAA	717
GTTCCCAGTATGGATCGGC	718
GTGTGCACTTCGCTTCACCT	719
GTGGTGGAATTTCTCTCAATT	720
GTGGACTTCTCTCAATTTTCT	721
GTGGACTTCTCTCAATTTTC	722
GTGCCTTCTCATCTGCCGGA	723
GTGCACTTCGCTTCACCTCT	724
GTGAAGCGAAGTGACACGG	725
GTCTGTGCCTTCTCATCTGC	726
GTCGGCGCTGAATCCTGCCG	727
GTCCCGTCGGCGCTGAATCC	728
GTATTGTGAGGATTCTTGTC	729
GGTGGACTTCTCTCAATTTT	730

HBV Target Sequence	SEQ ID NO:
GGGCGCACCTCTCTTTACGC	731
GGCTGTAGGCATAAAATTGGT	732
GGCGCTGAATCCTGCGGACG	733
GGAGGAACCTTCAGGGAAGG	734
GGACTCCCCGTCTGTGCCTT	735
GGACCGTGTGCACTTCGCTT	736
GCTGTAGGCATAAAATTGGT	737
GCTGAATCCTGCGGACGACC	738
GCTCAGTTTACTAGTGCCAT	739
GCGGACTCCCCGTCTGTGCC	740
GCGCTGAATCCTGCGGACGA	741
GCGCACCTCTCTTTACGCGG	742
GCCTTCTCATCTGCCGGACC	743
GCCGGACCGTGTGCACTTCG	744
GCCGATCCATACTGCGGAAC	745
GCCGATCCATACTGCGGAA	746
GCACTTCGCTTCACCTCTGC	747
GCACTAGTAACTGAGCCAG	748
GCACCTCTCTTTACGCGGAC	749
GATCCATACTGCGGAACTCC	750
GACTCCCCGTCTGTGCCTTC	751
GACCGTGTGCACTTCGCTTC	752
GACAAGAATCCTCACAATAC	753
GAATCCTCACAATACCGCAG	754
GAAAATTGAGAGAAGTCCAC	755
CTTTGTTTACGTCCCGTCGG	756
CTTGTTGACAAGAATCCTCACAATACCGCA	757
CTTCTCATCTGCCGGACCGT	758
CTGTGCCTTCTCATCTGCCG	759
CTGGCTCAGTTTACTAGTGC	760
CTGGCTCAGTTTACTAGTG	761
CTGCCGGACCGTGTGCACTT	762
CTGCCGATCCATACTGCGGA	763
CTCTGCCGATCCATACTGCG	764
CTCCTGGCTCAGTTTACTAG	765
CTCCCCGTCTGTGCCTTCTC	766
CTCACAATACCGCAGAGTCT	767
CTATCAAGGTATGTTGCCCGTTTGTCTCT	768
CGTGTGCACTTCGCTTCACC	769
CGTGGTGGACTTCTCTCAATTTTCTAGGGG	770

HBV Target Sequence	SEQ ID NO:
CGTGGTGGACTTCTCTCAATTTCT	771
CGTGGTGGACTTCTCTCAAT	772
CGTGGTGGACTTCTCTCAAT	773
CGTCGGCGCTGAATCCTGCG	774
CGTCCCGTCGGCGCTGAATC	775
CGGGGCGCACCTCTCTTTAC	776
CGGCGCTGAATCCTGCGGAC	777
CGGACTCCCCGTCTGTGCCT	778
CGGACCGTGTGCACTTCGCT	779
CGCTGAATCCTGCGGACGAC	780
CGCGGACTCCCCGTCTGTGC	781
CGCACCTCTCTTTACGCGGA	782
CCTTTGTTTACGTCCCGTCG	783
CCTTCTCATCTGCCGGACCG	784
CCTTAGGCACTCCTGCCTCT	785
CCTGGCTCAGTTTACTAGTG	786
CCTCTGCCGATCCATACTGCGGAACCTCCTA	787
CCTCTGCCGATCCATACTGC	788
CCTCTCTTTACGCGGACTCC	789
CCTCACAATACCGCAGAGTC	790
CCGTGTGCACTTCGCTTCAC	791
CCGTGTGCACTTCGCTGTTG	792
CCGTGTGCACTTCGCT	793
CCGTCTGTGCCTTCTCATCT	794
CCGTCGGCGCTGAATCCTGC	795
CCGGTCCGTGTGCACTTCGC	796
CCGGACCGTGTGCACTTCGC	797
CCGATCCATACTGCGGAACCT	798
CCCGTCGGCGCTGAATCCTG	799
CCCCGTCTGTGCCTTCTCAT	800
CAGGTCCCCTAGAAGAAGAA	801
CACGGGGCGCACCTCTCTTT	802
CACCTCTCTTTACGCGGACT	803
CAAGAATCCTCACAATACCG	804
ATCTGCCGGACCGTGTGCAC	805
ATCCTCACAATACCGCAGAG	806
AGACTCGTGGTGGACTTCTCTCAATTTCT	807
AGAATCCTCACAATACCGCA	808
ACGTCCCGTCGGCGCTGAAT	809
ACGCGGACTCCCCGTCTGTG	810

HBV Target Sequence	SEQ ID NO:
ACCTCTCTTTACGCGGACTC	811
ACCGTGTGCACTTCGCTTCA	812
ACCAATTTATGCCTACAGCG	813
ACAAGAATCCTCACAATACC	814
AATCCTCACAATACCGCAGA	815
AAGGTATGTTGCCCGTTTGT	816
AAGAATCCTCACAATACCGC	817
CTAGACTCGTGGTGGACTTC	819
CCTGCTGCTATGCCTCATCT	820
CTGCTGCTATGCCTCATCTT	821
TGCTGCTATGCCTCATCTTC	822
GCTGCTATGCCTCATCTTCT	823
CTGCTATGCCTCATCTTCTT	824
CCTATGGGAGTGGGCCTCAG	825
GCCATTTGTTCAAGTGGTTTCG	826
GGAGGCTGTAGGCATAAATT	827
GAGGCTGTAGGCATAAATTG	828
AGGCTGTAGGCATAAATTGG	829
GGCTGTAGGCATAAATTGGT	830
TTCAAGCCTCCAAGCTGTGC	831
TCAAGCCTCCAAGCTGTGCC	832
CAAGCCTCCAAGCTGTGCCT	833
AAGCCTCCAAGCTGTGCCTT	834
AGCCTCCAAGCTGTGCCTTG	835
GCCTCCAAGCTGTGCCTTGG	836
GAAGTCCCTCGCCTCGCAGA	837
CACCATATTCTTGGAACA	838

[0228] In some embodiments, the HBV target comprises a sequence of **SEQ ID NO: 1, 4, 675-838**. In some embodiments, the HBV target comprises a sequence of CTTGG TCATG GGCCA TCAG (**SEQ ID NO: 1**). In some embodiments, the HBV target comprises a sequence of GCACT TCGCT TCACC TCTGC (**SEQ ID NO: 4**).

[0229] In certain embodiments, the compounds provided herein 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.

[0230] In certain embodiments, the HBV target comprises the sequence recited in **SEQ ID NO: 3** or a portion thereof or a variant thereof. In certain embodiments, the modified oligonucleotide is at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or 100% complementary to a HBV nucleic acid.

[0231] In certain embodiments, the HBV target comprises the sequence recited in **SEQ ID NO:**

1. In certain embodiments, the modified oligonucleotide is at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or 100% complementary to **SEQ ID NO: 1**.

[0232] In certain embodiments, the HBV target comprises the sequence recited in **SEQ ID NO:**

4. In certain embodiments, the modified oligonucleotide is at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or 100% complementary to **SEQ ID NO: 4**.

[0233] In some embodiments, the compounds or compositions comprise a modified oligonucleotide of 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 or 50 linked nucleosides in length that bind to a HBV target sequence. In certain embodiments, the compounds or compositions comprise a modified oligonucleotide of 20 linked nucleosides that bind to a HBV target sequence.

[0234] In certain embodiments, the modified oligonucleotide of 20 linked nucleosides has a nucleobase sequence of GCAGA GGTGA AGCGA AGTGC (**SEQ ID NO: 2**). A chart showing the nucleobase positions of **SEQ ID NO: 2** is shown below:

<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>13</u>	<u>14</u>	<u>15</u>	<u>16</u>	<u>17</u>	<u>18</u>	<u>19</u>	<u>20</u>
G	C	A	G	A	G	G	T	G	A	A	G	C	G	A	A	G	T	G	C

[0235] In certain embodiments, the modified oligonucleotide of 20 linked nucleosides has a nucleobase sequence of GTGAA GCGAA GTGCA CACGG (**SEQ ID NO: 5**). A chart showing the nucleobase positions of **SEQ ID NO: 5** is shown below:

<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>13</u>	<u>14</u>	<u>15</u>	<u>16</u>	<u>17</u>	<u>18</u>	<u>19</u>	<u>20</u>
G	T	G	A	A	G	C	G	A	A	G	T	G	C	A	C	A	C	G	G

[0236] In certain embodiments, the compound comprises a modified oligonucleotide described herein. Exemplary modified oligonucleotides include but are not limited to those shown in **FIGS. 1-2**. In some embodiments, the compound comprises a modified oligonucleotide comprising the sequence of any one of **AUS1010 to AUS1714**. In some embodiments, the

compound comprises a modified oligonucleotide comprising the sequence of any one of **SEQ ID NOS: 11 to 666**, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto. [0237] In certain embodiments, the compound comprises a modified oligonucleotide comprising the sequence of any one of **A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW or AX**. Table 3 shows a correspondence table for the alphabet identifier, the AUS identifier and the SEQ ID NO corresponding to the sequence of the modified oligonucleotide.

Table 3. Nomenclature Correspondence Table for Modified Oligonucleotides

Alphabet Identifier	AUS Identifier	SEQ ID NO:
A	AUS1427/AUS1461	390/424
B	AUS1422	385
C	AUS1423	386
D	AUS1424	387
E	AUS1425	388
F	AUS1426	389
G	AUS1428	391
H	AUS1458	421
I	AUS1459	422
J	AUS1460	423
K	AUS1322	294
L	AUS1326	298
M	AUS1439	402
N	AUS1443	406
O	AUS1444	407
P	AUS1462	425
Q	AUS1499	462
R	AUS1500	463
S	AUS1465	428
T	AUS1469	432
U	AUS1475	438
V	AUS1473	436
W	AUS1472	435
X	AUS1474	437
Y	AUS1478	441
Z	AUS1479	442
AA	AUS1494	457
AB	AUS1463	426
AC	AUS1468	431
AD	AUS1466/AUS1495	429/458
AE	AUS1429	392

Alphabet Identifier	AUS Identifier	SEQ ID NO:
AF	AUS1431	394
AG	AUS1436	399
AH	AUS1437	400
AI	AUS1441	404
AJ	AUS1464	427
AK	AUS1467	430
AL	AUS1470	433
AM	AUS1471	434
AN	AUS1477	440
AO	AUS1476/AUS1493	439/456
AP	AUS1430	393
AQ	AUS1432	395
AR	AUS1440	403
AS	AUS1438	401
AT	AUS1489	452
AU	AUS1488/AUS1497	451/460
AV	AUS1490	453
AW	AUS1498	461
AX	AUS1433	396

Features of Modified Oligonucleotides

[0238] In certain embodiments, the compound comprises a modified oligonucleotide of 20 linked nucleosides consisting of: a first gap segment consisting of linked nucleosides; a second gap segment consisting of linked nucleosides; a separator segment consisting of a nucleoside linked to the first gap segment and the second gap segment; a 5' wing segment consisting of linked nucleosides; and a 3' wing segment consisting of linked nucleosides; wherein the first gap segment, separator segment, and second gap segment are positioned between the 5' wing segment and the 3' wing segment; wherein the first gap segment is attached to the 5' wing segment, and the second gap segment is attached to the 3' wing segment, wherein the first gap segment, separator segment, and second gap segment, taken together, consist of 8 or 9 or 10 or 11 or 12 linked nucleosides.

[0239] In certain embodiments, the compound comprises a modified oligonucleotide of 20 linked nucleosides consisting of: a first gap segment consisting of linked nucleosides; a second gap segment consisting of linked nucleosides; a separator segment consisting of a nucleoside linked between the first gap segment and the second gap segment; wherein the first gap segment, the separator segment, and the second gap segment, taken together, consist of 8 or 9 or 10 or 11

or 12 nucleotides; a 5' wing segment consisting of linked nucleosides; and a 3' wing segment consisting of linked nucleosides; wherein the first gap segment, second gap segment, and separator segment are positioned between the 5' wing segment and the 3' wing segment; wherein the first gap segment is attached to the 5' wing segment, and the second gap segment is attached to the 3' wing segment; wherein the first gap segment consists of linked nucleosides with 2'-deoxy sugars; wherein the second gap segment consists of linked nucleosides with 2'-deoxy sugars; wherein the separator segment consists of one nucleoside comprising a 2'-O(CH₂)₂-OCH₃ sugar or a 2'-OCH₃ sugar, wherein the modified oligonucleotide is at least 95% complementary to SEQ ID NO: 1; and wherein said modified oligonucleotide has a nucleobase sequence of SEQ ID NO: 2 (GCAGA GGTGA AGCGA AGTGC).

[0240] In certain embodiments, the compound comprises a modified oligonucleotide of 20 linked nucleosides consisting of: a first gap segment consisting of linked nucleosides; a second gap segment consisting of linked nucleosides; a separator segment consisting of a nucleoside linked between the first gap segment and the second gap segment; wherein the first gap segment, the separator segment, and the second gap segment, taken together, consist of 8 or 9 or 10 or 11 or 12 nucleotides; a 5' wing segment consisting of linked nucleosides; and a 3' wing segment consisting of linked nucleosides; wherein the first gap segment, second gap segment, and separator segment are positioned between the 5' wing segment and the 3' wing segment; wherein the first gap segment is attached to the 5' wing segment, and the second gap segment is attached to the 3' wing segment; wherein the first gap segment consists of linked nucleosides with 2'-deoxy sugars; wherein the second gap segment consists of linked nucleosides with 2'-deoxy sugars; wherein the separator segment consists of one nucleoside comprising a 2'-O(CH₂)₂-OCH₃ sugar or a 2'-OCH₃ sugar, wherein at least one of the nucleosides in the 5' wing segment comprises a 2'-O(CH₂)₂-OCH₃ sugar; and wherein at least one of the nucleosides in the 3' wing segment comprises a 2'-O(CH₂)₂-OCH₃ sugar; wherein the modified oligonucleotide is at least 95% complementary to SEQ ID NO: 1; and wherein said modified oligonucleotide has a nucleobase sequence of SEQ ID NO: 2 (GCAGA GGTGA AGCGA AGTGC).

A. 5' Wing Segment, General

[0241] In certain embodiments, at least one of the nucleosides in the 5' wing segment comprises a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, each of the nucleosides in the 5' wing segment comprises a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment comprises a nucleoside with a 2'-deoxy sugar. In certain embodiments, the 5' wing segment

comprises a nucleoside with a 2'-deoxy sugar, and the other nucleosides in the 5' wing segment comprise a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the nucleoside at position 2 of SEQ ID NO: 2 comprises a 2'-deoxy sugar. In certain embodiments, the nucleoside at position 3 of SEQ ID NO: 2 comprises a 2'-deoxy sugar. In certain embodiments, the nucleoside at position 4 of SEQ ID NO: 2 comprises a 2'-deoxy sugar. In certain embodiments, the 5' wing segment further comprises two nucleosides each with a 2'-deoxy sugar. In certain embodiments, the nucleosides at positions 2 and 3 of SEQ ID NO: 2 comprise a 2'-deoxy sugar. In certain embodiments, the nucleosides at positions 2 and 4 of SEQ ID NO: 2 comprise a 2'-deoxy sugar. In certain embodiments, the nucleosides at positions 3 and 4 of SEQ ID NO: 2 comprise a 2'-deoxy sugar. In certain embodiments, the nucleosides at positions 2 and 5 of SEQ ID NO: 2 comprise a 2'-deoxy sugar.

[0242] In certain embodiments, one, two, three, or four of the nucleosides in the 5' wing segment comprise a sugar modification described herein. In certain embodiments, one, two, three, or four of the nucleosides in the 5' wing segment comprise a bicyclic sugar. In certain embodiments, one, two, three, or four of the nucleosides in the 5' wing segment comprise a constrained ethyl sugar.

[0243] In certain embodiments, one, two, three, or four of the nucleosides in the 5' wing segment comprise a locked nucleic acid. In certain embodiments, one, two, three, or four of the nucleosides in the 5' wing segment comprise a 4'-CH₂-O-2' sugar. In certain embodiments, two of the nucleosides in the 5' wing segment comprise a locked nucleic acid. In certain embodiments, two of the nucleosides in the 5' wing segment comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the nucleosides at positions 1 and 3 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the nucleosides at positions 1 and 3 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

B1. 5' Wing Segment w/4 linked nucleosides

[0244] In certain embodiments, the 5' wing segment consists of 4 linked nucleosides. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein each of the 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein 3 of the 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein 2 of the 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment

consists of 4 linked nucleosides, wherein 1 of the 4 linked nucleosides comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

B2. 5' Wing Segment w/4 linked nucleosides, 2'-deoxy combinations

[0245] In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein one of the linked nucleosides comprises a nucleoside with a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein two of the linked nucleosides comprises a nucleoside with a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, one of the 4 linked nucleosides comprises a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, two of the 4 linked nucleosides comprise a locked nucleic acid, and the other linked nucleoside comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0246] In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein the nucleoside at position 2 of SEQ ID NO: 2 comprises a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein the nucleoside at position 2 of SEQ ID NO: 2 comprises a 2'-deoxy sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein the nucleoside at position 3 of SEQ ID NO: 2 comprises a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein the nucleoside at position 3 of SEQ ID NO: 2 comprises a 2'-deoxy sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein the nucleoside at position 4 of SEQ ID NO: 2 comprises a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein the nucleoside at position 4 of SEQ ID NO: 2 comprises a 2'-deoxy sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0247] In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein the nucleosides at positions 2 and 3 of SEQ ID NO: 2 comprise a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein the nucleosides at positions 2 and 3 of SEQ ID NO: 2 comprise a 2'-deoxy sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein the nucleosides at positions 2 and 4 of SEQ ID NO: 2 comprise a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein the nucleosides at positions 2 and 4 of SEQ ID NO: 2 comprise a 2'-deoxy sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein the nucleosides at positions 3 and 4 of SEQ ID NO: 2 comprise a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein the nucleosides at positions 3 and 4 of SEQ ID NO: 2 comprise a 2'-deoxy sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

B3. 5' Wing Segment w/4 linked nucleosides, 2'-LNA combinations

[0248] In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked nucleosides comprise a locked nucleic acid. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein two of the 4 linked nucleosides comprise a locked nucleic acid. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked nucleosides comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein two of the 4 linked nucleosides comprise a 4'-CH₂-O-2' sugar.

[0249] In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked nucleosides comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein two of the 4 linked nucleosides comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked nucleosides comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, wherein two of the 4

linked nucleosides comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0250] In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 1 and 3 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 1 and 3 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0251] In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 1 and 3 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 1 and 3 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

C1. 5' Wing Segment w/5 linked nucleosides

[0252] In certain embodiments, the 5' wing segment consists of 5 linked nucleosides. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein each of the 5 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein 4 of the 5 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein 3 of the 5 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein 2 of the 5 linked nucleosides comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein 1 of the 5 linked nucleosides comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

C2. 5' Wing Segment w/5 linked nucleosides, 2'-deoxy combinations

[0253] In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein one of the linked nucleosides comprises a nucleoside with a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein two of the linked nucleosides comprises a nucleoside with a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, and each of the other 4 linked nucleosides comprise a

nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, one of the 5 linked nucleosides comprises a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, two of the 5 linked nucleosides comprise a locked nucleic acid, and the other 2 linked nucleosides comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0254] In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein the nucleoside at position 2 of SEQ ID NO: 2 comprises a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein the nucleoside at position 2 of SEQ ID NO: 2 comprises a 2'-deoxy sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein the nucleoside at position 3 of SEQ ID NO: 2 comprises a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein the nucleoside at position 3 of SEQ ID NO: 2 comprises a 2'-deoxy sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein the nucleoside at position 4 of SEQ ID NO: 2 comprises a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein the nucleoside at position 4 of SEQ ID NO: 2 comprises a 2'-deoxy sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0255] In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein the nucleosides at positions 2 and 3 of SEQ ID NO: 2 comprise a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein the nucleosides at positions 2 and 3 of SEQ ID NO: 2 comprise a 2'-deoxy sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein the nucleosides at positions 2 and 4 of SEQ ID NO: 2 comprise a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein the nucleosides at positions 2 and 4 of SEQ ID NO: 2 comprise a 2'-deoxy sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5

linked nucleosides, wherein the nucleosides at positions 3 and 4 of SEQ ID NO: 2 comprise a 2'-deoxy sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein the nucleosides at positions 3 and 4 of SEQ ID NO: 2 comprise a 2'-deoxy sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

C3. 5' Wing Segment w/5 linked nucleosides, 2'-LNA combinations

[0256] In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprise a locked nucleic acid. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein two of the 5 linked nucleosides comprise a locked nucleic acid. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein two of the 5 linked nucleosides comprise a 4'-CH₂-O-2' sugar.

[0257] In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprise a locked nucleic acid, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein two of the 5 linked nucleosides comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprise a 4'-CH₂-O-2' sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, wherein two of the 5 linked nucleosides comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0258] In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 1 and 3 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 1 and 3 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0259] In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 1 and 3 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 5' wing segment consists of 5 linked nucleosides, and the nucleosides at

positions 1 and 3 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

D. 3' Wing Segment, General

[0260] In certain embodiments, at least one of the nucleosides in the 3' wing segment comprises a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, each of the nucleosides in the 3' wing segment comprise a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment comprises a nucleoside with a 2'-deoxy sugar. In certain embodiments, the 3' wing segment comprises a nucleoside with a 2'-deoxy sugar, and the other nucleosides in the 3' wing segment comprise a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment further comprises two nucleosides each with a 2'-deoxy sugar.

[0261] In certain embodiments, one, two, three, or four of the nucleosides in the 3' wing segment comprise a sugar modification described herein. In certain embodiments, one, two, three, or four of the nucleosides in the 3' wing segment comprise a bicyclic sugar. In certain embodiments, one, two, three, or four of the nucleosides in the 3' wing segment comprise a constrained ethyl sugar.

[0262] In certain embodiments, one, two, three, or four of the nucleosides in the 3' wing segment comprise a locked nucleic acid. In certain embodiments, one, two, three, or four of the nucleosides in the 3' wing segment comprise a 4'-CH₂-O-2' sugar. In certain embodiments, two of the nucleosides in the 3' wing segment comprise a locked nucleic acid. In certain embodiments, two of the nucleosides in the 3' wing segment comprise a 4'-CH₂-O-2' sugar. In certain embodiments, three of the nucleosides in the 3' wing segment comprise a locked nucleic acid. In certain embodiments, three of the nucleosides in the 3' wing segment comprise a 4'-CH₂-O-2' sugar.

[0263] In certain embodiments, the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the nucleosides at

positions 17 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the nucleosides at positions 19 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the nucleosides at positions 19 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0264] In certain embodiments, the nucleosides at positions 17, 18, and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the nucleosides at positions 17, 18, and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the nucleosides at positions 17, 19, and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the nucleosides at positions 17, 19, and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

E1. 3' Wing Segment w/4 linked nucleosides

[0265] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein each of the 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein 3 of the 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein 2 of the 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein 1 of the 4 linked nucleosides comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

E2. 3' Wing Segment w/4 linked nucleosides, 2'-deoxy combinations

[0266] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein one of the linked nucleosides comprises a nucleoside with a 2'-deoxy sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein two of the linked nucleosides comprises a nucleoside with a 2'-deoxy sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, one of the 4 linked nucleosides comprises a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, two of the 4 linked nucleosides comprise a locked nucleic acid, and the other linked nucleoside comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

E3. 3' Wing Segment w/4 linked nucleosides, 2'-LNA combinations

[0267] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked nucleosides comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein two of the 4 linked nucleosides comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked nucleosides comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein two of the 4 linked nucleosides comprise a 4'-CH₂-O-2' sugar.

[0268] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked nucleosides comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein two of the 4 linked nucleosides comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein three of the 4 linked nucleosides comprise a locked nucleic acid, and the other linked nucleoside comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein one of the 4 linked

nucleosides comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein two of the 4 linked nucleosides comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, wherein three of the 4 linked nucleosides comprise a 4'-CH₂-O-2' sugar, and the other linked nucleoside comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0269] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0270] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0271] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0272] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0273] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0274] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0275] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0276] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0277] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0278] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0279] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0280] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of

the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0281] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0282] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0283] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0284] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0285] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 19 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 19 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0286] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 19 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at

positions 19 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0287] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17, 18 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17, 18 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0288] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17, 18 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid, and the other linked nucleoside comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17, 18 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and the other linked nucleoside comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0289] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17, 19 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17, 19 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0290] In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17, 19 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and the other linked nucleoside comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 4 linked nucleosides, and the nucleosides at positions 17, 19 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and the other linked nucleoside comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

F1. 3' Wing Segment w/5 linked nucleosides

[0291] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein each of the 5 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein 4 of the 5 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein 3 of the 5 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein 2 of the 5 linked nucleosides comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5

linked nucleosides, wherein 1 of the 5 linked nucleosides comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

F2. 3' Wing Segment w/5 linked nucleosides, 2'-deoxy combinations

[0292] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein one of the linked nucleosides comprises a nucleoside with a 2'-deoxy sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein two of the linked nucleosides comprises a nucleoside with a 2'-deoxy sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, one of the 5 linked nucleosides comprises a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, two of the 5 linked nucleosides comprise a locked nucleic acid, and the other 2 linked nucleosides comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

F3. 3' Wing Segment w/5 linked nucleosides, 2'-LNA combinations

[0293] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein two of the 5 linked nucleosides comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein two of the 5 linked nucleosides comprise a 4'-CH₂-O-2' sugar.

[0294] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprise a locked nucleic acid, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein two of the 5 linked nucleosides comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5

linked nucleosides, wherein three of the 5 linked nucleosides comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein one of the 5 linked nucleosides comprise a 4'-CH₂-O-2' sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein two of the 5 linked nucleosides comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, wherein three of the 5 linked nucleosides comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0295] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0296] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0297] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0298] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0299] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain

embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0300] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0301] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0302] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0303] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0304] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0305] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0306] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0307] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0308] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0309] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0310] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0311] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 19 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 19 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0312] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 19 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of

the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 19 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0313] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17, 18, and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17, 18, and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0314] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17, 18, and 19 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17, 18 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0315] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17, 19, and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17, 19, and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0316] In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17, 19, and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 5 linked nucleosides, and the nucleosides at positions 17, 19 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 2 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

G1. 3' Wing Segment w/6 linked nucleosides

[0317] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein each of the 6 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein 5 of the 6 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein 4 of the 6 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment

consists of 6 linked nucleosides, wherein 3 of the 6 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein 2 of the 6 linked nucleosides comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein 1 of the 6 linked nucleosides comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

G2. 3' Wing Segment w/6 linked nucleosides, 2'-deoxy combinations

[0318] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein one of the linked nucleosides comprises a nucleoside with a 2'-deoxy sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein two of the linked nucleosides comprises a nucleoside with a 2'-deoxy sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein one of the 6 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, and each of the other 5 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein one of the 6 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, one of the 6 linked nucleosides comprises a locked nucleic acid, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein one of the 5 linked nucleosides comprises a nucleoside with a 2'-deoxy sugar, two of the 6 linked nucleosides comprise a locked nucleic acid, and the other 3 linked nucleosides comprises a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

G3. 3' Wing Segment w/6 linked nucleosides, 2'-LNA combinations

[0319] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein one of the 6 linked nucleosides comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein two of the 6 linked nucleosides comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein one of the 6 linked nucleosides comprise a 4'-CH₂-O-2' sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein two of the 6 linked nucleosides comprise a 4'-CH₂-O-2' sugar.

[0320] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein one of the 6 linked nucleosides comprise a locked nucleic acid, and each of the other 5 linked

nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein two of the 6 linked nucleosides comprise a locked nucleic acid, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein three of the 6 linked nucleosides comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein one of the 6 linked nucleosides comprise a 4'-CH₂-O-2' sugar, and each of the other 5 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein two of the 6 linked nucleosides comprise a 4'-CH₂-O-2' sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, wherein three of the 6 linked nucleosides comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0321] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0322] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 16 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0323] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0324] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at

positions 16 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0325] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0326] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 16 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0327] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0328] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0329] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0330] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0331] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0332] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0333] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0334] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 18 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0335] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0336] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 18 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0337] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 19 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain

embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 19 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0338] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 19 and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 19 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 4 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0339] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17, 18, and 19 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17, 18, and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0340] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17, 18, and 19 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17, 18 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

[0341] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17, 19, and 20 of SEQ ID NO: 2 comprise a locked nucleic acid. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17, 19, and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.

[0342] In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17, 19, and 20 of SEQ ID NO: 2 comprise a locked nucleic acid, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the 3' wing segment consists of 6 linked nucleosides, and the nucleosides at positions 17, 19 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar, and each of the other 3 linked nucleosides comprise a nucleoside with a 2'-O(CH₂)₂-OCH₃ sugar.

H-0. Gap Segments

[0343] In some embodiments, a modified oligonucleotide comprises or consists of at least one gap segment. In some embodiments, a modified oligonucleotide comprises or consists of at least two gap segments. In some embodiments, a modified oligonucleotide comprises 1, 2, 3, 4, 5, 6,

7, 8, or 9 or 10 gap segments. Gap segments are consecutively referred to a first, a second, a third, a fourth, a fifth, a sixth, a seventh, an eighth, a ninth or a tenth gap segment, respectively, with the first gap segment being nearest to the 5' end of the modified oligonucleotide and last gap segment being nearest to the 3' end of the modified oligonucleotide. In some embodiments, a modified oligonucleotide comprises or consists of 2 gap segments. In some embodiments, a modified oligonucleotide comprises or consists of 3 gap segments. In some embodiments, a modified oligonucleotide comprises or consists of 4 gap segments. In some embodiments, a modified oligonucleotide comprises or consists of 5 gap segments. In some embodiments, a modified oligonucleotide comprises or consists of 6 gap segments. In some embodiments, a modified oligonucleotide comprises or consists of 7 gap segments.

[0344] In some embodiments, a gap segment comprises or consists of 1 to 20 linked nucleosides. In some embodiments, the gap segment consists of 1 nucleoside. In some embodiments, the gap segment consists of 2 linked nucleosides. In some embodiments, the gap segment consists of 3 linked nucleosides. In some embodiments, the gap segment consists of 4 linked nucleosides. In some embodiments, the gap segment consists of 5 linked nucleosides. In some embodiments, the gap segment consists of 6 linked nucleosides. In some embodiments, the gap segment consists of 7 linked nucleosides. In some embodiments, the gap segment consists of 8 linked nucleosides. In some embodiments, the gap segment consists of 9 linked nucleosides. In some embodiments, the gap segment consists of 10 linked nucleosides.

H. First gap segment

[0345] In certain embodiments, the first gap segment comprises or consists of 1, 2, 3, 4, 5, 6, 7, or 8 linked nucleosides. In certain embodiments, the first gap segment comprises or consists of 4 or 5 linked nucleosides. In certain embodiments, the first gap segment comprises or consists of 4 linked nucleosides. In certain embodiments, the first gap segment comprises or consists of 5 linked nucleosides.

[0346] In certain embodiments, the first gap segment comprises or consists of 1, 2, 3, 4, 5, 6, 7, or 8 linked nucleosides, each with 2'-deoxy sugars. In certain embodiments, the first gap segment comprises or consists of 4 or 5 linked nucleosides, each with 2'-deoxy sugars. In certain embodiments, the first gap segment comprises or consists of 4 linked nucleosides, each with 2'-deoxy sugars. In certain embodiments, the first gap segment comprises or consists of 5 linked nucleosides, each with 2'-deoxy sugars.

[0347] In certain embodiments, the first gap segment, each with 2'-deoxy sugars, is positions 6, 7, 8, and 9 of SEQ ID NO: 2. In certain embodiments, the first gap segment, each with 2'-deoxy sugars, is positions 5, 6, 7, 8, and 9 of SEQ ID NO: 2. In certain embodiments, the first gap segment, each with 2'-deoxy sugars, is positions 6, 7, 8, 9, and 10 of SEQ ID NO: 2.

I. Second gap segment

[0348] In certain embodiments, the second gap segment comprises or consists of 1, 2, 3, 4, 5, 6, 7, or 8 linked nucleosides. In certain embodiments, the second gap segment comprises or consists of 4 or 5 linked nucleosides. In certain embodiments, the first gap segment comprises or consists of 4 linked nucleosides. In certain embodiments, the first gap segment comprises or consists of 5 linked nucleosides.

[0349] In certain embodiments, the second gap segment comprises or consists of 1, 2, 3, 4, 5, 6, 7, or 8 linked nucleosides, each with 2'-deoxy sugars. In certain embodiments, the second gap segment comprises or consists of 4 linked nucleosides, each with 2'-deoxy sugars. In certain embodiments, the second gap segment comprises or consists of 5 linked nucleosides, each with 2'-deoxy sugars. In certain embodiments, the second gap segment comprises or consists of 6 linked nucleosides, each with 2'-deoxy sugars.

[0350] In certain embodiments, the second gap segment, each with 2'-deoxy sugars, is positions 11, 12, 13, and 14 of SEQ ID NO: 2. In certain embodiments, the second gap segment, each with 2'-deoxy sugars, is positions 11, 12, 13, 14, and 15 of SEQ ID NO: 2. In certain embodiments, the second gap segment, each with 2'-deoxy sugars, is positions 11, 12, 13, 14, 15, and 16 of SEQ ID NO: 2.

J. Separator segment

[0351] In some embodiments, the separator segment comprises 0, 1, 2, 3, 4 or 5 linked nucleosides. In some embodiments, the separator segment comprises 1 nucleoside. In some embodiments, the nucleoside comprises a modification. Exemplary modifications of the nucleoside include but are not limited to a 2'-methoxyethyl nucleoside, a 2'-O-methyl nucleoside, a 2'-OH nucleoside, a 2'-fluoro 2'-deoxy nucleoside, a 2'-F-arabinonucleic acid (2'-F-ANA), a glycol nucleic acid (GNA) or a locked nucleic acid (LNA).

[0352] In certain embodiments, the separator segment consists of one nucleoside comprising a 2'-OCH₃ sugar. In certain embodiments, the separator segment consists of one nucleoside comprising a 2'-O(CH₂)₂-OCH₃ sugar.

[0353] In certain embodiments, the separator segment is at position 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 of SEQ ID NO: 2. In certain embodiments, the separator segment is at position 9 or 10 or 11 of SEQ ID NO: 2. In certain embodiments, the separator segment is at position 10 of SEQ ID NO: 2.

[0354] In certain embodiments, the separator segment is at position 10 of SEQ ID NO: 2 and consists of one nucleoside comprising a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the separator segment consists of one nucleoside comprising a 2'-OCH₃ sugar. In certain embodiments, the separator segment is at position 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 of SEQ ID NO: 2. In certain embodiments, the separator segment is at position 9 or 10 or 11 of SEQ ID NO: 2. In certain embodiments, the separator segment is at position 10 of SEQ ID NO: 2.

[0355] In certain embodiments, the separator segment consists of one nucleoside comprising a 2'-O(CH₂)₂-OCH₃ sugar. In certain embodiments, the separator segment consists of one nucleoside comprising a 2'-OCH₃ sugar. In certain embodiments, the separator segment is at position 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 of SEQ ID NO: 2. In certain embodiments, the separator segment is at position 9 or 10 or 11 of SEQ ID NO: 2. In certain embodiments, the separator segment is at position 10 of SEQ ID NO: 2.

K. Oligonucleotide backbone

[0356] In certain embodiments, the modified oligonucleotide is a single-stranded modified oligonucleotide. In certain embodiments, at least one internucleoside linkage is a modified internucleoside linkage. In certain embodiments, each internucleoside linkage is a phosphorothioate internucleoside linkage.

L. Modified nucleobases

[0357] In certain embodiments, the modified oligonucleotide comprises at least one modified nucleobase. In certain embodiments, wherein the modified oligonucleotide comprises one, two, or three modified nucleobase(s). In certain embodiments, the modified nucleobase is a 5-methylcytosine. In certain embodiments, the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine.

M1. 5-4-1-5-5 modified oligonucleotides

[0358] In certain embodiments, the modified oligonucleotide comprises: a 5' wing segment consisting of 5 linked nucleosides; a first gap segment consisting of 4 linked nucleosides with

2'-deoxy sugars; a separator segment at position 10 of SEQ ID NO:2; a second gap segment consisting of 5 linked nucleosides with 2'-deoxy sugars; and a 3' wing segment consisting of 5 linked nucleosides. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that a cytosine at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine and further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage.

[0359] In certain embodiments, the modified oligonucleotide comprises: a 5' wing segment described in Section C1, C2, or C3; a first gap segment consisting of 4 linked nucleosides with 2'-deoxy sugars; a separator segment at position 10 of SEQ ID NO: 2; a second gap segment consisting of 5 linked nucleosides with 2'-deoxy sugars; and a 3' wing segment described in Section F1, F2, or F3. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises a cytosine at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine and further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage.

[0360] In certain embodiments, the modified oligonucleotide comprises a sequence which is A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, or Z.

M2. 4-5-1-5-5 modified oligonucleotides

[0361] In certain embodiments, the modified oligonucleotide comprises: a 5' wing segment consisting of 4 linked nucleosides; a first gap segment consisting of 5 linked nucleosides with 2'-deoxy sugars; a separator segment at position 10 of SEQ ID NO:2; a second gap segment consisting of 5 linked nucleosides with 2'-deoxy sugars; and a 3' wing segment consisting of 5 linked nucleosides. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine. In an exemplary embodiment, the modified oligonucleotide of this

paragraph further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that a cytosine at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine and further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage.

[0362] In certain embodiments, the modified oligonucleotide comprises: a 5' wing segment described in Section B1, B2, or B3; a first gap segment consisting of 5 linked nucleosides with 2'-deoxy sugars; a separator segment at position 10 of SEQ ID NO:2; a second gap segment consisting of 5 linked nucleosides with 2'-deoxy sugars; and a 3' wing segment described in Section F1, F2, or F3. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises a cytosine at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine and further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage.

[0363] In certain embodiments, the modified oligonucleotide comprises a sequence which is AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, or AO.

[0364] Exemplary annotations of the positions and segments of AO (AUS 1493 or SEQ ID NO: 456) is shown below.

		5' Wing segment				First Gap segment					Separator segment	Second Gap segment					3' Wing segment				
Position		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
SEQ ID NO	AUS #																				
456	AUS1493	G	C	A	G	a	g	g	t	g	A	a	g	c	g	a	A	+G	T	+G	+C

M3. 5-4-1-6-4 modified oligonucleotides

[0365] In certain embodiments, the modified oligonucleotide comprises: a 5' wing segment consisting of 5 linked nucleosides; a first gap segment consisting of 4 linked nucleosides with 2'-deoxy sugars; a separator segment at position 10 of SEQ ID NO:2; a second gap segment consisting of 6 linked nucleosides with 2'-deoxy sugars; and a 3' wing segment consisting of 4 linked nucleosides. In an exemplary embodiment, the modified oligonucleotide of this

paragraph further comprises that the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that a cytosine at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine and further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage.

[0366] In certain embodiments, the modified oligonucleotide comprises: a 5' wing segment described in Section C1, C2, or C3; a first gap segment consisting of 4 linked nucleosides with 2'-deoxy sugars; a separator segment at position 10 of SEQ ID NO:2; a second gap segment consisting of 6 linked nucleosides with 2'-deoxy sugars; and a 3' wing segment described in Section E1, E2, or E3. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises a cytosine at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine and further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage.

[0367] In certain embodiments, the modified oligonucleotide comprises a sequence which is AP, AQ, AR, or AS.

M4. 4-5-1-4-6 modified oligonucleotides

[0368] In certain embodiments, the modified oligonucleotide comprises: a 5' wing segment consisting of 4 linked nucleosides; a first gap segment consisting of 5 linked nucleosides with 2'-deoxy sugars; a separator segment at position 10 of SEQ ID NO:2; a second gap segment consisting of 4 linked nucleosides with 2'-deoxy sugars; and a 3' wing segment consisting of 6 linked nucleosides. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that a cytosine at positions 2, 13, and 20 of SEQ ID NO: 2 are each

5-methylcytosine and further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage.

[0369] In certain embodiments, the modified oligonucleotide comprises: a 5' wing segment described in Section B1, B2, or B3; a first gap segment consisting of 5 linked nucleosides with 2'-deoxy sugars; a separator segment at position 10 of SEQ ID NO:2; a second gap segment consisting of 4 linked nucleosides with 2'-deoxy sugars; and a 3' wing segment described in Section G1, G2, or G3. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises a cytosine at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine and further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage.

[0370] In certain embodiments, the modified oligonucleotide comprises a sequence which is AT or AU.

M5. 5-4-1-4-6 modified oligonucleotides

[0371] In certain embodiments, the modified oligonucleotide comprises: a 5' wing segment consisting of 5 linked nucleosides; a first gap segment consisting of 4 linked nucleosides with 2'-deoxy sugars; a separator segment at position 10 of SEQ ID NO:2; a second gap segment consisting of 4 linked nucleosides with 2'-deoxy sugars; and a 3' wing segment consisting of 6 linked nucleosides. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that a cytosine at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine and further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage.

[0372] In certain embodiments, the modified oligonucleotide comprises: a 5' wing segment described in Section C1, C2, or C3; a first gap segment consisting of 4 linked nucleosides with 2'-deoxy sugars; a separator segment at position 10 of SEQ ID NO:2; a second gap segment

consisting of 4 linked nucleosides with 2'-deoxy sugars; and a 3' wing segment described in Section G1, G2, or G3. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises a cytosine at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine and further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage.

[0373] In certain embodiments, the modified oligonucleotide comprises a sequence which is AV or AW.

M6. 4-5-1-6-4 modified oligonucleotides

[0374] In certain embodiments, the modified oligonucleotide comprises: a 5' wing segment consisting of 4 linked nucleosides; a first gap segment consisting of 5 linked nucleosides with 2'-deoxy sugars; a separator segment at position 10 of SEQ ID NO:2; a second gap segment consisting of 6 linked nucleosides with 2'-deoxy sugars; and a 3' wing segment consisting of 4 linked nucleosides. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that a cytosine at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine and further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage.

[0375] In certain embodiments, the modified oligonucleotide comprises: a 5' wing segment described in Section B1, B2, or B3; a first gap segment consisting of 5 linked nucleosides with 2'-deoxy sugars; a separator segment at position 10 of SEQ ID NO:2; a second gap segment consisting of 6 linked nucleosides with 2'-deoxy sugars; and a 3' wing segment described in Section E1, E2, or E3. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises that the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises internucleoside linkages in which each is a phosphorothioate

internucleoside linkage. In an exemplary embodiment, the modified oligonucleotide of this paragraph further comprises a cytosine at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine and further comprises internucleoside linkages in which each is a phosphorothioate internucleoside linkage.

[0376] In certain embodiments, the modified oligonucleotide comprises a sequence which is AX.

[0377] 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.

[0378] In certain embodiments, the compounds or compositions comprise a salt of the modified oligonucleotide.

[0379] In certain embodiments, the compounds or compositions further comprise a pharmaceutically acceptable carrier or diluent.

[0380] In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% complementary to a HBV nucleic acid, as measured over the entirety of the modified oligonucleotide. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% complementary to SEQ ID NO: 1, as measured over the entirety of the modified oligonucleotide.

[0381] In certain embodiments, the compound or modified oligonucleotide is single-stranded.

[0382] 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-CH₃ group.

[0383] 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'-(CH₂)-O-2' bridge,

wherein n is 1 or 2. In certain embodiments, the bicyclic sugar comprises a 4'-CH₂-O-2' bridge. In certain embodiments, the bicyclic sugar comprises a 4'-CH(CH₃)-O-2' bridge.

Methods

[0384] The disclosure provides methods of treating a subject with an HBV infection, or with an HBV-related disease, disorder, or condition, the methods comprising administering a therapeutically effective amount of the modified oligonucleotides described herein, or a pharmaceutical composition comprising same. In certain embodiments, the modified oligonucleotide comprises a sequence of any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto.

[0385] In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same inhibit HBV mRNA expression in the subject. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same inhibit HBV DNA levels in the subject. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same inhibit HBV protein levels and/or antigen levels in the subject. The modified oligonucleotides of the disclosure, when administered to a subject, can reduce the levels of HBV mRNA, DNA, or protein, including HBV antigens such as, but not limited to HBsAg and HbeAg.

[0386] The disclosure provides methods for treating a HBV-related disease, disorder, and/or condition in an subject, the methods comprising administering a therapeutically effective amount of any pharmaceutical composition as described above to a subject in need thereof, so as to treat the HBV-related diseases, disorders, and condition. In certain embodiments, the subject is a mammal. In certain embodiments, the subject 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).

[0387] In certain embodiments, the modified oligonucleotides target a region of a HBV nucleic acid. In certain embodiments, such modified 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 of the HBV nucleic acid. 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.

[0388] Certain embodiments provide methods of treating a HBV related disease, disorder, or condition in a subject, comprising administering to the subject in need thereof a modified oligonucleotide, for example a modified oligonucleotide comprising any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto, or pharmaceutical composition described herein.

[0389] Certain embodiments provide a method of reducing HBV expression in a subject comprising administering to the subject a modified oligonucleotide, for example a modified oligonucleotide comprising any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto, or a pharmaceutical composition described herein.

[0390] Certain embodiments provide a method of preventing, ameliorating or treating an HBV-related disease, disorder or condition in a subject comprising administering to the animal a modified oligonucleotide, for example a modified oligonucleotide comprising any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto, or a pharmaceutical composition described herein.

[0391] 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, and HBV viremia. HBV-related conditions or disorders can have 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, which 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 indicate an HBV-related condition or disorder.

[0392] Certain embodiments provide a method of reducing HBV mRNA expression in a subject comprising administering to the subject a modified oligonucleotide, for example a modified oligonucleotide comprising any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto, or pharmaceutical composition described herein. In certain embodiments, reduction of HBV mRNA expression in the subject prevents,

ameliorates or treats an HBV-related disease, disorder or condition. In certain embodiments, reduction of HBV mRNA expression in the subject ameliorates or treats HBV infection. In certain embodiments, reduction of HBV mRNA expression in the subject 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%.

[0393] Certain embodiments provide a method of reducing HBV protein levels in an subject comprising administering to the subject a modified oligonucleotide, for example a modified oligonucleotide comprising any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto, or a pharmaceutical composition described herein. In certain embodiments, reduction of HBV protein levels in the subject prevents, ameliorates or treats an HBV-related disease, disorder or condition. In certain embodiments, reduction of HBV protein levels in an subject ameliorates or treats HBV infection. In certain embodiments, reduction of HBV protein levels in the subject 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%.

[0394] Certain embodiments provide a method of reducing HBV DNA levels in a subject comprising administering to the subject a modified oligonucleotide, for example a modified oligonucleotide comprising any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto, or a pharmaceutical composition described herein. In certain embodiments, reduction of HBV DNA levels in the subject prevents, ameliorates or treats an HBV-related disease, disorder or condition. In certain embodiments, the subject may be a mammal such as a 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 a subject ameliorates or treats HBV infection. In certain embodiments, reduction of HBV DNA levels in a subject 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%.

[0395] Certain embodiments provide a method of reducing HBV antigen levels in a subject comprising administering to the subject a modified oligonucleotide, for example a modified oligonucleotide comprising any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto, or a pharmaceutical composition described herein. In certain embodiments, the antigen is HBsAg or HBeAg. In certain embodiments, reduction of HBV antigen levels in the subject prevents, ameliorates or treats an HBV-related disease, disorder or condition. In certain embodiments, reduction of HBV antigen levels in the subject 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%.

[0396] Certain embodiments provide a method of reducing HBV DNA and HBV antigen in a subject infected with a hepatitis B virus, comprising administering to the subject a modified oligonucleotide, for example a modified oligonucleotide comprising any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto, or a pharmaceutical composition described herein. 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.

[0397] Certain embodiments provide a method for treating a subject with a HBV related disease, disorder or condition comprising: a) identifying said subject with the HBV related disease, disorder or condition, and b) administering to said subject a therapeutically effective amount of a modified oligonucleotide or pharmaceutical composition described herein. In certain embodiments, the therapeutically effective amount of the modified oligonucleotide or pharmaceutical composition administered to the subject treats or reduces the HBV related disease, disorder or condition, or a symptom thereof, in the subject. 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 such as hepatocellular carcinoma, liver inflammation, liver fibrosis, liver cirrhosis, liver failure, diffuse hepatocellular inflammatory disease, hemophagocytic syndrome, serum hepatitis, HBV viremia, liver disease-related to transplantation, or any combination thereof.

[0398] Certain embodiments provide a method for treating a subject with a HBV related disease, disorder or condition comprising: a) identifying said subject with the HBV related disease, disorder or condition, and b) administering to said subject a therapeutically effective amount of a modified oligonucleotide or pharmaceutical composition described herein. In certain embodiments, the therapeutically effective amount of the modified oligonucleotide or pharmaceutical composition administered to the subject treats or reduces the HBV related disease, disorder or condition, or a symptom thereof, in the subject. 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.

[0399] In certain embodiments, HBV has the sequence as set forth in GenBank Accession Numbers U95551.1 (**SEQ ID NO: 3**) or any variant or fragment thereof. In certain embodiments, HBV has the sequence set forth in **SEQ ID NOS: 667-674**.

[0400] In certain embodiments, the subject is a human.

[0401] In certain embodiments, the subject is a monkey, for example a cynomolgus monkey.

[0402] In certain embodiments, the subject is a rodent, such as a mouse or rat.

[0403] In certain embodiments, the modified oligonucleotides or pharmaceutical 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 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 first agent and one or more second agents are not co-administered.

[0404] 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 specific embodiments, the disease comprises liver cancer, and the one or more second agents comprises a chemotherapeutic agent, such as Gemcitabine (Gemzar), Oxaliplatin (Eloxatin), Cisplatin, Doxorubicin, 5-Fluorouracil, Capecitabine (Xeloda), or Mitoxantrone (Novantrone). In specific embodiments, the disease comprises liver disease, and

the one or more second agents comprise corticosteroids, diuretics, beta-blockers, or a combination thereof.

[0405] Modified oligonucleotides of the disclosure, and pharmaceutical compositions comprising same, can be administered to the subject by any suitable route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), intraperitoneal (into the body cavity) and transmucosal administration.

[0406] In certain embodiments, administration comprises parenteral administration. In certain embodiments, administration comprises subcutaneous administration. In certain embodiments, administration comprises intravenous injection or infusion.

[0407] Certain embodiments provide a method for reducing an amount of HBV mRNA, DNA, protein and/or an amount of HBV antigen in a subject infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a pharmaceutical composition as described above to the subject 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 subject before treatment. In some embodiments, the subject 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).

[0408] The disclosure provides methods for reducing an amount of HBV mRNA, DNA, protein and/or an amount of HBV antigen, or a combination thereof, in a subject infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a modified oligonucleotide or pharmaceutical composition comprising same as described above to the subject so as to reduce the hepatitis B virus infection and the hepatitis B antigen, compared to the amount of HBV mRNA, protein and/or an amount of HBV antigen in the subject before treatment. In certain embodiments, the amount of mRNA is reduced at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% compared to the amount before administration of the modified antisense oligonucleotide or pharmaceutical composition. In certain embodiments, the amount of mRNA is reduced at least 50% compared to the amount before administration of the modified

antisense oligonucleotide or pharmaceutical composition. In certain embodiments, the amount of mRNA is reduced at least 60% compared to the amount before administration of the modified antisense oligonucleotide or pharmaceutical composition. In certain embodiments, the amount of mRNA is reduced at least 70% compared to the amount before administration of the modified antisense oligonucleotide or pharmaceutical composition. In certain embodiments, the amount of mRNA is reduced at least 80% compared to the amount before administration of the modified antisense oligonucleotide or pharmaceutical composition. In certain embodiments, the amount of mRNA is reduced at least 90% compared to the amount before administration of the modified antisense oligonucleotide or pharmaceutical composition.

[0409] The disclosure provides methods for reducing an amount of HBV mRNA, DNA, protein and/or an amount of HBV antigen in a subject infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a modified oligonucleotide or pharmaceutical composition as described above to the subject so as to reduce the hepatitis B virus infection and the hepatitis B antigen, compared to the amount of HBV virus, mRNA, DNA, protein and/or an amount of HBV antigen in the subject before treatment, wherein the amount of mRNA is reduced at least 75% compared to the amount before administration of the modified oligonucleotide or pharmaceutical composition. In certain embodiments, the methods reduce an amount of HBV virus, mRNA, DNA, protein and/or an amount of HBV antigen in a subject infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a modified oligonucleotide or pharmaceutical composition as described above to the subject so as to reduce the hepatitis B virus infection and the hepatitis B antigen, compared to the amount of hepatitis B virus and HBV antigen in the subject before treatment, wherein the amount of mRNA is reduced at least 80% compared to the amount before administration of the modified oligonucleotide or pharmaceutical composition. In certain embodiments, the methods reduce an amount of HBV virus, mRNA, DNA, protein and/or an amount of HBV antigen in a subject infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a modified oligonucleotide or pharmaceutical composition as described above to the subject so as to reduce the hepatitis B virus infection and the hepatitis B antigen, compared to the amount of HBV virus, mRNA, DNA protein and/or an amount of HBV antigen in the subject before treatment, wherein the amount of mRNA is reduced at least 85% compared to the amount before administration of the modified oligonucleotide or pharmaceutical composition. In certain embodiments, the methods reduce an

amount of HBV virus, mRNA, DNA, protein and/or an amount of HBV antigen in a subject infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a modified oligonucleotide or pharmaceutical composition as described above to the subject so as to reduce the hepatitis B virus infection and the hepatitis B antigen, compared to the amount of HBV virus, mRNA, DNA, protein and/or an amount of HBV antigen in the subject before treatment, wherein the amount of mRNA is reduced at least 90% compared to the amount before administration of the modified oligonucleotide or pharmaceutical composition. In certain embodiments, the methods reduce an amount of HBV virus, mRNA, DNA, protein and/or an amount of HBV antigen in a subject infected with a hepatitis B virus, the method comprising administering a therapeutically effective amount of a modified oligonucleotide or pharmaceutical composition as described above to the subject so as to reduce the hepatitis B virus infection, mRNA, protein and/or the hepatitis B antigen, compared to the amount of HBV mRNA, protein and/or an amount of HBV antigen in the subject before treatment, wherein the amount of mRNA is reduced at least 95% compared to the amount before administration of the modified oligonucleotide or pharmaceutical composition. 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.

[0410] The disclosure provides methods for promoting seroconversion of a hepatitis B virus in a mammal infected with HBV, the method comprising administering a therapeutically effective amount of a modified oligonucleotide, for example a modified oligonucleotide comprising any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto, or pharmaceutical composition as described above to a subject infected with hepatitis B; monitoring for presence of HBeAg plus HBeAb in a serum sample of the subject, or monitoring for presence of HBsAg in a serum sample of the subject, 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 subject.

[0411] Certain embodiments provide the use of a modified oligonucleotide, for example a modified oligonucleotide comprising any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto, or a pharmaceutical composition as described herein for preventing, ameliorating or treating liver disease, or symptom thereof, in an subject.

[0412] In some embodiments, EC₅₀ of cells treated with a modified oligonucleotide (*e.g.*, SEQ ID NO: 11-666) was measured as a proxy for the effectiveness of the modified oligonucleotide. In some embodiments, the EC₅₀ is about 0.1 nM to about 250 nM. In some embodiments, the EC₅₀ is 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, less than 45 nM, less than 44 nM, less than 43 nM, less than 42 nM, less than 41 nM, less than 40 nM, less than 39 nM, less than 38 nM, less than 37 nM, less than 36 nM, less than 35 nM, less than 34 nM, less than 33 nM, less than 32 nM, less than 31 nM, less than 30 nM, less than 29 nM, less than 28 nM, less than 27 nM, less than 26 nM, less than 25 nM, less than 24 nM, less than 23 nM, less than 22 nM, less than 21 nM, less than 20 nM, less than 19 nM, less than 18 nM, less than 17 nM, less than 16 nM, less than 15 nM, less than 14 nM, less than 13 nM, less than 12 nM, less than 11 nM, less than 10 nM, less than 9 nM, less than 8 nM, less than 7 nM, less than 6 nM, less than 5 nM, less than 4 nM, less than 3 nM, less than 2 nM, less than 1 nM, less than 0.9 nM, less than 0.8 nM, less than 0.7 nM, less than 0.6 nM, less than 0.5 nM, less than 0.4 nM, less than 0.3 nM, less than 0.2 nM or less than 0.1 nM.

[0413] In some embodiments, the ratio of EC₅₀ of cells treated with a modified oligonucleotide (*e.g.*, SEQ ID NO: 11-666) / EC₅₀ of cells treated with a reference oligonucleotide (*e.g.*, SEQ ID NO: 10) is calculated as a measure of efficacy in reducing HBsAg levels. In some embodiments, the ratio is about 0.05 to about 250. In some embodiments, the EC₅₀ ratio is less than 250, less than 150, less than 100, less than 90, less than 80, less than 70, less than 65, less than 60, less than 55, less than 50, less than 49, less than 47, less than 46, less than 45, less than 44, less than 43, less than 42, less than 41, less than 40, less than 39, less than 38, less than 37, less than 36, less than 35, less than 34, less than 33, less than 32, less than 31, less than 30, less than 29, less than 28, less than 27, less than 26, less than 25, less than 24, less than 23, less than 22, less than 21, less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less

than 7, less than 6, less than 5, less than 4, less than 3, less than 2, less than 1, less than 0.9, less than 0.8, less than 0.7, less than 0.6, less than 0.5, less than 0.4, less than 0.3, less than 0.2 or less than 0.1.

[0414] In certain embodiments, the compounds or compositions as described herein are efficacious by virtue of having at least one of an *in vitro* IC₅₀ 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.

[0415] In certain embodiments, the compounds or compositions as described herein are efficacious by virtue of having at least one of an *in vitro* IC₅₀ 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.

[0416] In certain embodiments, the compounds or compositions as described herein are efficacious by virtue of having at least one of an *in vitro* IC₅₀ 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.

[0417] In some embodiments, the MTT CC25 value (nM) is used as a measure of cellular toxicity. In some embodiments the MTT CC25 of cells treated with the modified oligonucleotide described herein is about 10 nM to about 250 nM. In some embodiments the MTT CC25 is greater than 10 nM, greater than 15 nM, greater than 20 nM, greater than 25 nM, greater than 30 nM, greater than 35 nM, greater than 40 nM, greater than 45 nM, greater than 50 nM, greater than 55 nM, greater than 60 nM, greater than 65 nM, greater than 70 nM, greater than 75 nM, greater than 80 nM, greater than 85 nM, greater than 90 nM, greater than 95 nM, greater than 100 nM, greater than 110 nM, greater than 120 nM, greater than 130 nM, greater than 140 nM, greater than 150 nM, greater than 160 nM, greater than 170 nM, greater than 180 nM, greater than 190 nM, greater than 200 nM, greater than 210 nM, greater than 220 nM, greater than 230 nM, greater than 240 nM or greater than 250 nM.

[0418] In some embodiments, the CCK8 CC30 (nM) is used as a measure of cellular toxicity. In some embodiments, the CCK8 CC30 of cells treated with the modified oligonucleotide

described herein is about 10 nM to about 250 nM. In some embodiments, the CCK8 CC30 is greater than 10 nM, greater than 15 nM, greater than 20 nM, greater than 25 nM, greater than 30 nM, greater than 35 nM, greater than 40 nM, greater than 45 nM, greater than 50 nM, greater than 55 nM, greater than 60 nM, greater than 65 nM, greater than 70 nM, greater than 75 nM, greater than 80 nM, greater than 85 nM, greater than 90 nM, greater than 95 nM, greater than 100 nM, greater than 110 nM, greater than 120 nM, greater than 130 nM, greater than 140 nM, greater than 150 nM, greater than 160 nM, greater than 170 nM, greater than 180 nM, greater than 190 nM, greater than 200 nM, greater than 210 nM, greater than 220 nM, greater than 230 nM, greater than 240 nM or greater than 250 nM.

[0419] In some embodiments, the “C/E ratio” is used as a measure of efficacy in reducing HBsAg levels relative to cellular toxicity. In some embodiments, the C/E ratio is calculated by taking the ratio of MTT CC25 (nM) of a modified oligonucleotide/ MTT CC25 (nM) of a reference oligonucleotide (e.g., SEQ ID NO: 10) and dividing it by the ratio of HBsAg EC₅₀ of a modified oligonucleotide (e.g., SEQ ID NO: 11-666) / HBsAg EC₅₀ of a reference oligonucleotide (e.g., SEQ ID NO: 10). In some embodiments, the C/E ratio is calculated by taking the ratio of CCK8 CC25 (nM) of a modified oligonucleotide/ CCK8 CC25 (nM) of a reference oligonucleotide (e.g., SEQ ID NO: 10) and dividing it by the ratio of HBsAg EC₅₀ of a modified oligonucleotide (e.g., SEQ ID NO: 11-666) / HBsAg EC₅₀ of a reference oligonucleotide (e.g., SEQ ID NO: 10). In some embodiments, the C/E ratio is about 0.01 to about 50. In some embodiments, the C/E ratio is greater than 1, greater than 1.1, greater than 1.2, greater than 1.3, greater than 1.4, greater than 1.5, greater than 1.6, greater than 1.7, greater than 1.8, greater than 1.9, greater than 2.0, greater than 2.1, greater than 2.2, greater than 2.3, greater than 2.4, greater than 2.5, greater than 2.6, greater than 2.7, greater than 2.8, greater than 2.9, greater than 3.0, greater than 10, greater than 11, greater than 12, greater than 13, greater than 14, greater than 15, greater than 16, greater than 17, greater than 18, greater than 19, greater than 20, greater than 21, greater than 22, greater than 23, greater than 24, greater than 25, greater than 30, greater than 35, greater than 40, greater than 45 or greater than 50.

[0420] 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.

[0421] In some embodiments, animals treated with the compounds or compositions comprising the modified oligonucleotides (e.g., SEQ ID NOS: 11-666) have a ALT level that is about 1.1 fold to about 10 fold decreased relative to an animal treated with a reference compound (e.g., SEQ ID NO: 10). In some embodiments, animals treated with the compounds or compositions comprising the modified oligonucleotides (e.g., SEQ ID NOS: 11-666) have a ALT level that is at least 1.25 fold decreased relative to an animal treated with a reference compound (e.g., SEQ ID NO: 10). In some embodiments, animals treated with the compounds or compositions comprising the modified oligonucleotides (e.g., SEQ ID NOS: 11-666) have a ALT level that is are 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1.7 fold, 1.8 fold, 1.9 fold, 2.0 fold, 2.1 fold, 2.2 fold, 2.3, fold, 2.4 fold, 2.5 fold, 2.6 fold, 2.7 fold, 2.8 fold, 2.9 fold, 3.0 fold, 3.1 fold, 3.2 fold, 3.3 fold, 3.4 fold, 3.5 fold, 3.6 fold, 3.7 fold, 3.8 fold, 3.9 fold, 4.0 fold, 4.1 fold, 4.2 fold, 4.3 fold, 4.4 fold, 4.5 fold, 4.6 fold, 4.7 fold, 4.8 fold, 4.9 fold or 5.0 fold decreased relative to an animal treated with a reference compound (e.g., SEQ ID NO: 10).

[0422] In some embodiments, animals treated with the compounds or compositions comprising the modified oligonucleotides (e.g., SEQ ID NOS: 11-666) have a AST level that is about 1.1 fold to about 10 fold decreased relative to an animal treated with a reference compound (e.g., SEQ ID NO: 10). In some embodiments, animals treated with the compounds or compositions comprising the modified oligonucleotides (e.g., SEQ ID NOS: 11-666) have a AST level that is at least 1.25 fold decreased relative to an animal treated with a reference compound (e.g., SEQ ID NO: 10). In some embodiments, animals treated with the compounds or compositions comprising the modified oligonucleotides (e.g., SEQ ID NOS: 11-666) have a AST level that is are 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1.7 fold, 1.8 fold, 1.9 fold, 2.0 fold, 2.1 fold, 2.2 fold, 2.3, fold, 2.4 fold, 2.5 fold, 2.6 fold, 2.7 fold, 2.8 fold, 2.9 fold, 3.0 fold, 3.1 fold, 3.2 fold, 3.3 fold, 3.4 fold, 3.5 fold, 3.6 fold, 3.7 fold, 3.8 fold, 3.9 fold, 4.0 fold, 4.1 fold, 4.2 fold, 4.3 fold, 4.4 fold, 4.5 fold, 4.6 fold, 4.7 fold, 4.8 fold, 4.9 fold or 5.0 fold decreased relative to an animal treated with a reference compound (e.g., SEQ ID NO: 10).

[0423] Certain embodiments provide the use of a modified oligonucleotide, for example a modified oligonucleotide comprising any one of SEQ ID NOS: 11-666, or a pharmaceutical

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

[0424] Certain embodiments provide the use of a modified oligonucleotide, for example a modified oligonucleotide comprising any one of SEQ ID NOS: 11-666, or a pharmaceutical composition as described herein for the manufacture of a medicament for treating, ameliorating, delaying or preventing liver disease in an animal.

Kits and Articles of Manufacture

[0425] The disclosure provides kits comprising the modified oligonucleotides described herein, and pharmaceutical compositions comprising same. In some embodiments, the modified oligonucleotide comprises a sequence of any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto.

[0426] 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, for example a composition comprising a modified oligonucleotide comprising a sequence of any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5 modifications thereto; 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.

[0427] Compositions comprising modified oligonucleotides can be lyophilized before being packaged in the kit, or can be provided in solution with a pharmaceutically acceptable carrier, diluent or excipient.

[0428] In certain embodiments, the kit further comprises at least one additional agent for the treatment of an HBV-related disease, disorder or condition.

Dosing and Administration

[0429] The disclosure provides methods comprising administering a therapeutically effective dose of the modified oligonucleotides, for example a modified oligonucleotide comprising any one of SEQ ID NOS: 11-666, or a modified oligonucleotide comprising 1, 2, 3, 4, or 5

modifications thereto, or a pharmaceutical composition comprising same, to a subject in need thereof.

[0430] In certain embodiments, a therapeutically effective dose of the for example a modified oligonucleotides, or pharmaceutical compositions comprising same, comprises a dose at which a maximum log₁₀ serum HBsAg reduction of at least -0.2, at least -0.3, at least -0.4, at least -0.5, at least -0.7, at least -1.0, at least -1.5, at least -2.0, at least -2.5, at least -3.0, at least -3.5, at least at least -4.0, or at least -4.5 is observed. In some embodiments, the reduction in maximum log₁₀ serum HBsAg is observed 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 days after administration of the therapeutically effective dose.

[0431] In certain embodiments, a therapeutically effective dose of the for example a modified oligonucleotides, or pharmaceutical compositions comprising same, comprises a dose that reduces a level of HbsAg in serum of the subject by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99%. In some embodiments, the reduction in serum HBsAg is observed 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 days after administration of the therapeutically effective dose.

[0432] In certain embodiments, a therapeutically effective dose of the for example a modified oligonucleotides, or pharmaceutical compositions comprising same, comprises a dose at which a maximum log₁₀ serum HBeAg reduction of at least -0.2, at least -0.3, at least -0.4, at least -0.5, at least -0.7, at least -1.0, at least -1.5, at least -2.0, at least -2.5, at least -3.0, at least -3.5, at least at least -4.0, or at least -4.5 is observed. In some embodiments, the reduction in maximum log₁₀ serum HBeAg is observed 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 days after administration of the therapeutically effective dose.

[0433] In certain embodiments, a therapeutically effective dose of the for example a modified oligonucleotides, or pharmaceutical compositions comprising same, comprises a dose that reduces a level of HBeAg in serum of the subject by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99%. In some embodiments, the reduction in serum HBeAg is observed 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 days after administration of the therapeutically effective dose.

[0434] In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 10 mg to 1500 mg, 10 mg to 1000 mg, 10 mg to 900 mg, 10 mg to 800 mg, 10 mg to 700 mg, 10 mg to 600 mg, 10 mg to 500 mg, 10 mg to 400 mg, 10 mg to 300 mg, 10 mg to 200 mg, 10 mg to 100 mg, 50 mg to 1500 mg, 50 mg to 1000 mg, 50 mg to 900 mg, 50 mg to 800 mg, 50 mg to 700 mg, 50 mg to 600 mg, 50 mg to 500 mg, 50 mg to 400 mg, 50 mg to 300 mg, 50 mg to 200 mg, 50 mg to 100 mg, 100 mg to 1500 mg, 100 mg to 1000 mg, 100 mg to 900 mg, 100 mg to 800 mg, 100 mg to 700 mg, 100 mg to 600 mg, 100 mg to 500 mg, 100 mg to 400 mg, 100 mg to 300 mg, 100 mg to 200 mg, 200 mg to 1500 mg, 200 mg to 1000 mg, 200 mg to 900 mg, 200 mg to 800 mg, 200 mg to 700 mg, 200 mg to 600 mg, 200 mg to 500 mg, 200 mg to 400 mg, 200 mg to 300 mg, 300 mg to 1500 mg, 300 mg to 1000 mg, 300 mg to 900 mg, 300 mg to 800 mg, 300 mg to 700 mg, 300 mg to 600 mg, 300 mg to 500 mg, or 300 mg to 400 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 50 mg to 1500 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 50 mg to 1000 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 50 mg to 700 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 50 mg to 500 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 50 mg to 450 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 50 mg to 300 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 50 mg to 200 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 100 mg to 1500 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 100 mg to

1000 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 100 mg to 700 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 100 mg to 500 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 100 mg to 450 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 100 mg to 300 mg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject in a dose ranging from 100 mg to 200 mg of the modified oligonucleotides.

[0435] In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at a dose ranging from 0.01 mg/kg to 30.0 mg/kg, 0.01 mg/kg to 27.0 mg/kg, 0.01 mg/kg to 25.0 mg/kg, 0.01 mg/kg to 22.0 mg/kg, 0.01 mg/kg to 20.0 mg/kg, 0.01 mg/kg to 15.0 mg/kg, 0.01 mg/kg to 10.0 mg/kg, 0.01 mg/kg to 8.0 mg/kg, 0.01 mg/kg to 5.0 mg/kg, 0.01 mg/kg to 4.0 mg/kg, 0.01 mg/kg to 3.0 mg/kg, 0.01 mg/kg to 2.0 mg/kg, 0.01 mg/kg to 1.0 mg/kg, 0.01 mg/kg to 0.5 mg/kg, 0.01 mg/kg to 0.1 mg/kg, 0.1 mg/kg to 30.0 mg/kg, 0.1 mg/kg to 27.0 mg/kg, 0.1 mg/kg to 25.0 mg/kg, 0.1 mg/kg to 22.0 mg/kg, 0.1 mg/kg to 20.0 mg/kg, 0.1 mg/kg to 18.0 mg/kg, 0.1 mg/kg to 15.0 mg/kg, 0.1 mg/kg to 12.0 mg/kg, 0.1 mg/kg to 10.0 mg/kg, 0.1 mg/kg to 8.0 mg/kg, 0.1 mg/kg to 5.0 mg/kg, 0.1 mg/kg to 4.0 mg/kg, 0.1 mg/kg to 3.0 mg/kg, 0.1 mg/kg to 2.0 mg/kg, 0.1 mg/kg to 1.0 mg/kg, 0.1 mg/kg to 0.5 mg/kg, 1.0 mg/kg to 30.0 mg/kg, 1.0 mg/kg to 27.0 mg/kg, 1.0 mg/kg to 25.0 mg/kg, 1.0 mg/kg to 22.0 mg/kg, 1.0 mg/kg to 20.0 mg/kg, 1.0 mg/kg to 18.0 mg/kg, 1.0 mg/kg to 15.0 mg/kg, 1.0 mg/kg to 12.0 mg/kg, 1.0 mg/kg to 10.0 mg/kg, 1.0 mg/kg to 8.0 mg/kg, 1.0 mg/kg to 5.0 mg/kg, 1.0 mg/kg to 4.0 mg/kg, 1.0 mg/kg to 3.0 mg/kg, 1.0 mg/kg to 2.0 mg/kg, 5.0 mg/kg to 30.0 mg/kg, 5.0 mg/kg to 27.0 mg/kg, 5.0 mg/kg to 25.0 mg/kg, 5.0 mg/kg to 22.0 mg/kg, 5.0 mg/kg to 20.0 mg/kg, 5.0 mg/kg to 18.0 mg/kg, 5.0 mg/kg to 15.0 mg/kg, 5.0 mg/kg to 10.0 mg/kg, 5.0 mg/kg to 7.0 mg/kg, 10.0 mg/kg to 30.0 mg/kg, 10.0 mg/kg to 27.0 mg/kg, 10.0 mg/kg to 25.0 mg/kg, 10.0 mg/kg to 22.0 mg/kg, 10.0 mg/kg to 20.0 mg/kg, 10.0 mg/kg to 18.0 mg/kg, 10.0 mg/kg to 15.0 mg/kg, 15.0 mg/kg to 30.0 mg/kg, 15.0 mg/kg to 25.0 mg/kg, 15.0 mg/kg to 20.0 mg/kg, 20 mg/kg to 30 mg/kg or 25 mg/kg to 30 mg/kg of the modified oligonucleotides. In

certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at a dose ranging from 0.01 mg/kg to 30 mg/kg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at a dose ranging from 0.1 mg/kg to 25 mg/kg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at a dose ranging from 1.0 mg/kg to 20 mg/kg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at a dose ranging from 5.0 mg/kg to 20 mg/kg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at a dose ranging from 1.0 mg/kg to 15 mg/kg of the modified oligonucleotides. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at a dose ranging from 1.0 mg/kg to 5.0 mg/kg of the modified oligonucleotides.

[0436] In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject daily, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, every 7 days, every 8 days, every 9 days, every 10 days, every 11 days, every 12 days, every 13 days, every 2 weeks, every 3 weeks, every 4 weeks, every 5 weeks, every 6 weeks, every 7 weeks, every 8 weeks, every 9 weeks, every 10 weeks, every 11 weeks, every 12 weeks, every 13 weeks, every 2 months, every 3 months or every 4 months.

[0437] In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject daily, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, every 7 days, every 8 days, every 9 days, every 10 days, every two weeks, every three weeks or monthly.

[0438] In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject for at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 1 year, at least 1.5 years, at least 2 years, at least 3 years, at least 4 years, or at least 5 years.

[0439] In certain embodiments, modified oligonucleotides or pharmaceutical compositions comprising same are administered to the subject until a specific outcome is achieved, for

example the level of HBsAg and/or HbeAg in the serum of the subject is reduced. In certain embodiments, administration of the modified oligonucleotides or pharmaceutical compositions comprising same to the subject continues until the HBsAg and/or HbeAg in the serum of the subject is reduced by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% compared to before the administration of the modified oligonucleotides or pharmaceutical compositions comprising same.

[0440] In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject once a day, twice a day, three times a day or four times a day.

[0441] In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every day. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every two days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every three days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every four days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every five days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every six days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every seven days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every eight days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every nine days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every ten days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every eleven days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every twelve days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every thirteen days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject

every two weeks. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every three weeks. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every month. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every 2 months. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every 3 months. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every 4 months. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every 5 months. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject every 6 months. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject two or more times a year. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject two or more times every two years. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject two or more times every two or more years.

[0442] In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject once every 1-30 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject once every 1-22 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject once every 1-15 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject once every 1-8 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject once every 1-5 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject once every 1-4 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject once every 1-3 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject once every 10-20 days. In certain embodiments, the modified

oligonucleotides or pharmaceutical compositions comprising same are administered to a subject once every 5-15 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to a subject once every 15-30 days.

[0443] In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 36 hours. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 48 hours. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 60 hours. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 72 hours. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 84 hours. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 96 hours. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 5 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 6 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 7 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 8-10 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 10-12 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 12-15 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 15-25 days. In certain embodiments, the modified oligonucleotides or pharmaceutical compositions comprising same are administered at least once every 20-30 days.

[0444] In some embodiments, administration of the modified oligonucleotides or pharmaceutical compositions comprising same comprises a dosing holiday. For example, the modified oligonucleotides or pharmaceutical compositions comprising same are administered to the subject every three days, followed by a week, two weeks, three weeks or a month with no administration, followed by a resumption of dosing. The person of ordinary skill in the art will

understand that this dosing holiday is exemplary. Dosing holidays of other duration and frequency are contemplated as within the scope of the instant disclosure. As a further example, the modified oligonucleotides or pharmaceutical compositions comprising same may be administered to the subject until levels of HBsAg or HbeAg fall below a certain threshold, or no HBV infection is detected in the subject, followed by a dosing holiday, and resumption of dosing if HBsAg or HbeAg in the serum of the subject is again detected.

[0445] In certain embodiments, a single one time dose of the modified oligonucleotides or pharmaceutical compositions comprising same is administered to a subject. In other embodiments, multiple doses of the modified oligonucleotides or pharmaceutical compositions comprising same are administered to the subject.

[0446] In certain embodiments, administration of the modified oligonucleotides or pharmaceutical compositions comprising same can comprise a dosing schedule in which the modified oligonucleotides or pharmaceutical compositions comprising same are administered more frequently initially, followed by less frequent doses. Such a dosing schedule has the advantage in that it can be used to maintain a steady state liver concentration of the modified oligonucleotides described herein. For example, if the liver half-life of the modified oligonucleotide is 3-4 weeks, an initial dosing schedule comprising loading doses on days 1 and 4, followed by weekly maintenance doses starting on day 8 (e.g., day 8, 15 and 22 etc.) can be used to achieve a steady state liver concentration of the modified oligos of the disclosure.

[0447] In certain embodiments, administration of the modified oligonucleotides or pharmaceutical compositions comprising same comprises a loading dose, followed by a loading maintenance dose.

[0448] The term “loading dose” refers to one or more doses of the modified oligonucleotides or pharmaceutical compositions that are administered in addition to a dosage regimen, or that are higher than the remaining doses in the dosing regimen. As used herein a “loading dose” may refer to one or more doses of the modified oligonucleotides or pharmaceutical compositions which are the same concentration or a lower concentration (if administered in addition to the regular dosage regimen), or a higher concentration than the doses of the modified oligonucleotides or pharmaceutical compositions that are administered as part of a dosage regimen (if administered in place of the regular dosage regimen). Loading doses may also be administered more frequently than maintenance doses in a dosing regimen.

[0449] The term “maintenance dose” as used herein refers to the repeated, regular administration of a therapeutic agent. The maintenance dose as used herein does not include a loading dose, which may, in some embodiments, be administered in addition, *e.g.*, prior, to the maintenance dose.

[0450] In an exemplary dosing regimen comprising a loading dose and a maintenance dose, the modified oligonucleotides or pharmaceutical compositions are administered at a higher loading dose, followed by a lower maintenance dose. For example, the loading dose can be about 1.1X, 1.2X, 1.3X, 1.4X, 1.5X, 1.6X, 1.7X, 1.8X, 1.9X, 2.0X, 2.1X, 2.2X, 2.3X, 2.4X, 2.5X, 3.0X, 3.5X, 4.0X, 4.5X or 5X higher than the maintenance dose. Alternatively, or in addition, the loading dose can be administered more frequently than the maintenance dose. For example, the loading dose can be administered every day, every 2 days, every 3 days, every 4 days, or every 5 days, for at least 1, 2, 3, 4 or 5 doses, followed by a maintenance dose every week, every 10 days, every 14 days and the like. The skilled artisan will appreciate that, should the need arise, a subject who has been dosed according to the maintenance dosing schedule can be dosed again with the loading dose or doses. As a further exemplary dosing schedule, a subject can be administered loading doses on days 1 and 4, followed by weekly maintenance doses on day 8.

Antisense compounds

[0451] 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 capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

[0452] 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.

[0453] 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.

[0454] 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.

[0455] 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.

[0456] Gautschi *et al.* 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*.

[0457] 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

[0458] 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.

[0459] 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.

[0460] 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-CH₃, 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.

[0461] 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.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

[0462] In certain embodiments, nucleotide sequences that encode HBV include, without limitation, the following: GENBANK Accession U95551.1 (incorporated herein as SEQ ID NO: 1).

[0463] 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.

[0464] 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.

[0465] 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.

[0466] 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 embodiments, 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 preceding 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.

[0467] 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 specifically exclude a certain structurally defined region such as the start codon or stop codon.

[0468] 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).

[0469] 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

[0470] 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.

[0471] 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.

[0472] 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

[0473] 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).

[0474] 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).

[0475] 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.

[0476] 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).

[0477] 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.

[0478] 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.

[0479] 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.

[0480] 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

[0481] The antisense compounds provided herein may also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or compound, 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.

[0482] As used herein, “complementary” polynucleotides are those that are capable of base pairing according to the standard Watson-Crick complementarity rules. Specifically, purines will base pair with pyrimidines to form a combination of guanine paired with cytosine (G:C) and adenine paired with either thymine (A:T) in the case of DNA, or adenine paired with uracil (A:U) in the case of RNA. For example, the sequence “A-G-T” binds to the complementary sequence “T-C-A.” It is understood that two polynucleotides may hybridize to each other even if they are not completely complementary to each other, provided that each has at least one region that is substantially complementary to the other.

[0483] As used herein, the terms “substantially complementary” or “partially complementary” mean that two nucleic acid sequences are complementary at least at about 50%, 60%, 70%, 80% or 90% of their nucleotides.

[0484] In some embodiments, the two nucleic acid sequences can be complementary at least at 85%, 90%, 95%, 96%, 97%, 98%, 99% or more of their nucleotides. In some embodiments, the two nucleic acid sequences can be between 60% to 100% complementary, between 70% to 100% complementary, between 80% and 100% complementary, between 90% and 100% complementary, between 60% to 90% complementary, between 60% to 80% complementary, between 60% and 70% complementary, between 70% and 90% complementary, between 70% and 80% complementary, between 80% and 100% complementary, or between 80% and 90% complementary.

[0485] The terms “substantially complementary” and “partially complementary” can also mean that two nucleic acid sequences can hybridize under high stringency conditions, and such conditions are well known in the art.

[0486] As used herein, the term “identity” means that sequences are compared with one another as follows. In order to determine the percentage identity of two nucleic acid sequences, the

sequences can first be aligned with respect to one another in order subsequently to make a comparison of these sequences possible. For this e.g. gaps can be inserted into the sequence of the first nucleic acid sequence and the nucleotides can be compared with the corresponding position of the second nucleic acid sequence. If a position in the first nucleic acid sequence is occupied by the same nucleotide as is the case at a position in the second sequence, the two sequences are identical at this position. The percentage identity between two sequences is a function of the number of identical positions divided by the number of all the positions compared in the sequences investigated.

[0487] A “percent identity” for aligned segments of a test sequence and a reference sequence is the percent of identical components which are shared by the two aligned sequences divided by the total number of components in reference sequence segment, i.e., the entire reference sequence or a smaller defined part of the reference sequence.

[0488] The percentage identity of two sequences can be determined with the aid of a mathematical algorithm. A preferred, but not limiting, example of a mathematical algorithm which can be used for comparison of two sequences is the algorithm of Karlin et al. (1993), PNAS USA, 90:5873-5877. Such an algorithm is integrated in the NBLAST program, with which sequences which have a desired identity to the sequences of the present invention can be identified. In order to obtain a gapped alignment, as described here, the “Gapped BLAST” program can be used, as is described in Altschul et al. (1997), Nucleic Acids Res, 25:3389-3402. If BLAST and Gapped BLAST programs are used, the preset parameters of the particular program (e.g. NBLAST) can be used. The sequences can be aligned further using version 9 of GAP (global alignment program) of the “Genetic Computing Group” using the preset (BLOSUM62) matrix (values -4 to +11) with a gap open penalty of -12 (for the first zero of a gap) and a gap extension penalty of -4 (for each additional successive zero in the gap). After the alignment, the percentage identity is calculated by expressing the number of agreements as a percentage content of the nucleic acids in the sequence claimed. The methods described for determination of the percentage identity of two nucleic acid sequences can also be used correspondingly, if necessary, on the coded amino acid sequences.

[0489] Useful methods for determining sequence identity are also disclosed in Guide to Huge Computers (Martin J. Bishop, ed., Academic Press, San Diego (1994)), and Carillo, H., and Lipton, D., (Applied Math48:1073(1988)). More particularly, preferred computer programs for determining sequence identity include but are not limited to the Basic Local Alignment Search

Tool (BLAST) programs which are publicly available from National Center Biotechnology Information (NCBI) at the National Library of Medicine, National Institute of Health, Bethesda, Md. 20894; see BLAST Manual, Altschul et al., NCBI, NLM, NIH; (Altschul et al., J. Mol. Biol. 215:403-410 (1990)); version 2.0 or higher of BLAST programs allows the introduction of gaps (deletions and insertions) into alignments; for peptide sequence BLASTX can be used to determine sequence identity; and, for polynucleotide sequence BLASTN can be used to determine sequence identity. Percent identity can be 70% identity or greater, e.g., at least 70% identity, at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 98% identity, at least 99% identity or 100% identity.

Modifications

[0490] 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.

[0491] 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.

[0492] 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

[0493] The naturally occurring 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.

[0494] 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.

[0495] 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

[0496] 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 substituent 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)(R²) (R = H, C₁-C₁₂ 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).

[0497] Examples of nucleosides having modified sugar moieties include, without limitation, nucleosides comprising 5'-vinyl, 5'-methyl (R or S), 4'-S, 2'-F, 2'-OCH₃, and 2'-O(CH₂)₂OCH₃ substituent groups. The substituent at the 2' position can also be selected from allyl, amino,

azido, thio, O-allyl, O-C₁-C₁₀ alkyl, OCF₃, O(CH₂)₂SCH₃, O(CH₂)₂-O-N(R^m)(Rⁿ), and O-CH₂-C(=O)-N(R^m)(Rⁿ), where each R^m and Rⁿ is, independently, H or substituted or unsubstituted C₁-C₁₀ alkyl.

[0498] 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'-(CH₂)-O-2' (LNA); 4'-(CH₂)-S-2'; 4'-(CH₂)₂-O-2' (ENA); 4'-CH(CH₃)-O-2' (cEt) and 4'-CH(CH₂OCH₃)-O-2', and analogs thereof (*see*, U.S. Patent 7,399,845, issued on July 15, 2008); 4'-C(CH₃)(CH₃)-O-2', and analogs thereof (*see*, published PCT International Application WO2009/006478, published January 8, 2009); 4'-CH₂-N(OCH₃)-2', and analogs thereof (*see*, published PCT International Application WO2008/150729, published December 11, 2008); 4'-CH₂-O-N(CH₃)-2' (*see*, published U.S. Patent Application US2004/0171570, published September 2, 2004); 4'-CH₂-N(R)-O-2', wherein R is H, C₁-C₁₂ alkyl, or a protecting group (*see*, U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH₂-C(H)(CH₃)-2' (*see*, Chattopadhyaya, *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂-C(=CH₂)-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 Invers. 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).

[0499] 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

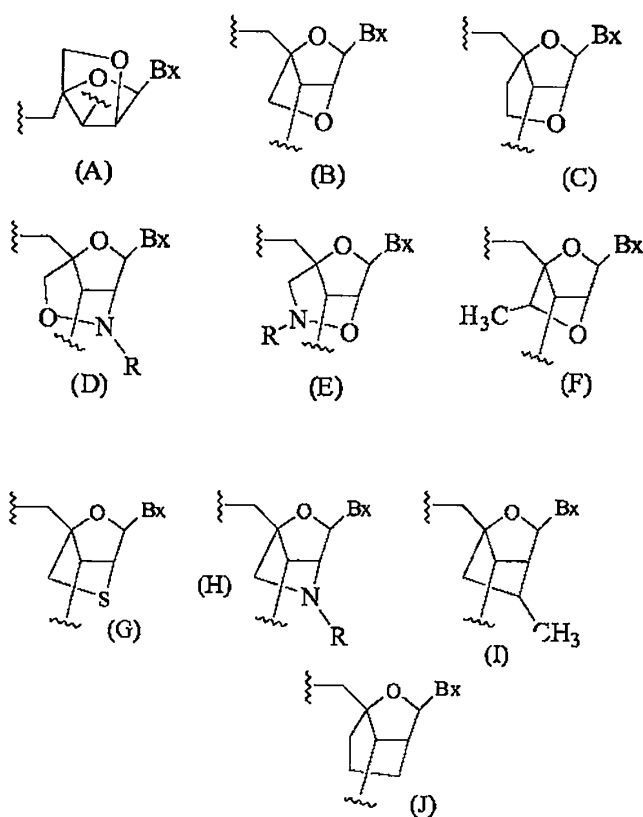
each J^1 and J^2 is, independently, H, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, acyl ($C(=O)-H$), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1 - C_{12} aminoalkyl, substituted C_1 - C_{12} aminoalkyl, or a protecting group.

[0500] In certain embodiments, the bridge of a bicyclic sugar moiety is, $-[C(R^a)(R^b)]_n-$, $-[C(R^a)(R^b)]_n-O-$, $-C(R^a)(R^b)-N(R)-O-$ or $-C(R^a)(R^b)-O-N(R)-$. In certain embodiments, the bridge is 4'-CH₂-2', 4'-(CH₂)₂-2', 4'-(CH₂)₃-2', 4'-CH₂-O-2', 4'-(CH₂)₂-O-2', 4'-CH₂-O-N(R)-2', and 4'-CH₂-N(R)-O-2', wherein each R is, independently, H, a protecting group, or C_1 - C_{12} alkyl.

[0501] 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'-CH₂-O-2') BNAs have been incorporated into antisense oligonucleotides that showed antisense activity (Frieden *et al.*, *Nucleic Acids Research*, 2003, 21, 63 6563 72).

[0502] In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α -L-Methyleneoxy (4'-CH₂-O-2') BNA, (B) β -D-Methyleneoxy (4'-CH₂-O-2') BNA, (C)

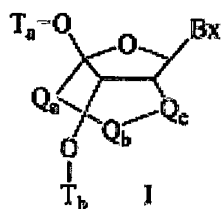
Ethyleneoxy (4'-(CH₂)₂-O-2') BNA, (D) Aminoxy (4'-CH₂-O-N(R)-2') BNA, (E) Oxyamino (4'-CH₂-N(R)-O-2') BNA, (F) Methyl(methyleneoxy) (4'-CH(CH₃)-O-2') BNA, (G) methylene-



thio (4'-CH₂-S-2') BNA, (H) methylene-amino (4'-CH₂-N(R)-2') BNA, (I) methyl carbocyclic (4'-CH₂-CH(CH₃)-2') BNA, and (J) propylene carbocyclic (4'-(CH₂)₃-2') BNA as depicted below.

wherein Bx is the base moiety and R is, independently, H, a protecting group or C₁-C₁₂ alkyl.

[0503] In certain embodiments, the bicyclic nucleoside is according to Formula I:



wherein:

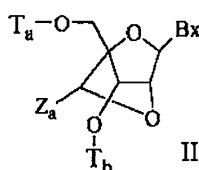
Bx is a heterocyclic base moiety;

$-Q^a-Q^b-Q^c-$ is $-\text{CH}_2-\text{N}(\text{R}^c)-\text{CH}_2-$, $-\text{C}(=\text{O})-\text{N}(\text{R}^c)-\text{CH}_2-$, $-\text{CH}_2-\text{O}-\text{N}(\text{R}^c)-$, $-\text{CH}_2-\text{N}(\text{R}^c)-\text{O}-$, or $-\text{N}(\text{R}^c)-\text{O}-\text{CH}_2$;

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.

[0504] In certain embodiments, bicyclic nucleoside having Formula II:



wherein:

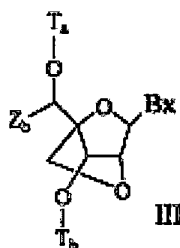
Bx is a heterocyclic base moiety;

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.

[0505] 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 , $\text{OC}(=\text{X})\text{J}^c$, and $\text{NJ}^c\text{C}(=\text{X})\text{NJ}^c\text{J}^d$, 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 .

[0506] In certain embodiments, bicyclic nucleoside having Formula III:



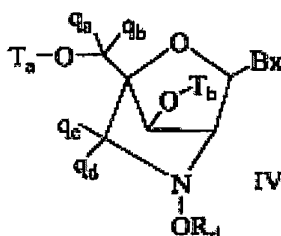
wherein:

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 C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, substituted C₁-C₆ alkyl, substituted C₂-C₆ alkenyl, substituted C₂-C₆ alkynyl, or substituted acyl (C(=O)-).

[0507] In certain embodiments, bicyclic nucleoside having Formula IV:



wherein:

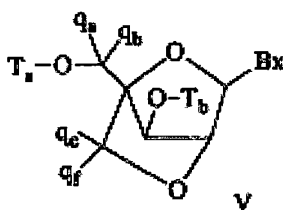
Bx is a heterocyclic base moiety;

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 C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl, or substituted C₂-C₆ alkynyl;

each q^a, q^b, q^c and q^d is, independently, H, halogen, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl, or substituted C₂-C₆ alkynyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, acyl, substituted acyl, C₁-C₆ aminoalkyl, or substituted C₁-C₆ aminoalkyl;

[0508] In certain embodiments, bicyclic nucleoside having Formula 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^c and q^d are each, independently, hydrogen, halogen, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl,

C₁-C₁₂ alkoxy, substituted C₁-C₁₂ alkoxy, OJ^j, SJ^j, SOJ^j, SO₂J^j, NJJ^k, N₃, CN, C(=O)OJ^j, C(=O)NJJ^k, C(=O)J^j, O-C(=O)NJJ^k, N(H)C(=NH)NJJ^k, N(H)C(=O)NJJ^k or N(H)C(=S)NJJ^k;

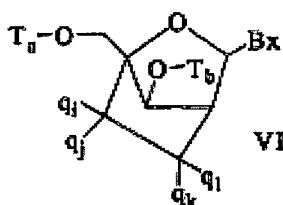
or q^e and q^f together are =C(q^g)(q^h);

q^g and q^h are each, independently, H, halogen, C₁-C₁₂ alkyl, or substituted C₁-C₁₂ alkyl.

[0509] The synthesis and preparation of the methyleneoxy (4'-CH₂-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.

[0510] Analogs of methyleneoxy (4'-CH₂-O-2') BNA, methyleneoxy (4'-CH₂-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 conformationally 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'-methyldamino-BNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

[0511] In certain embodiments, bicyclic nucleoside having Formula 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 qⁱ, q^j, q^k and q^l is, independently, H, halogen, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy, substituted C₁-C₁₂ alkoxy, OJ^j, SJ^j, SOJ^j, SO₂J^j, NJJ^k, N₃, CN, C(=O)OJ^j,

$C(=O)NJ^jJ^k$, $C(=O)J^j$, $O-C(=O)NJ^jJ^k$, $N(H)C(=NH)NJ^jJ^k$, $N(H)C(=O)NJ^jJ^k$, or $N(H)C(=S)NJ^jJ^k$; and

q^i and q^j or q^i 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.

[0512] One carbocyclic bicyclic nucleoside having a 4'-(CH₂)₃-2' bridge and the alkenyl analog, bridge 4'-CH=CH-CH₂-2', 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).

[0513] 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.

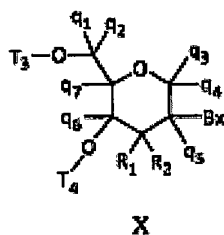
[0514] As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting the 2' carbon atom and the 4' carbon atom.

[0515] As used herein, "monocyclic 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.

[0516] 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; SCH₃; OCN; Cl; Br; CN; CF₃; OCF₃; SOCH₃; SO₂CH₃; ONO₂; NO₂; N₃; NH₂; 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, modified 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).

[0517] 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), mannitol nucleic acid (MNA) (*see* Leumann, CJ. *Bioorg. & Med. Chem.* (2002) 10:841-854), fluoro HNA (F-HNA), or those compounds having Formula X: wherein independently for each of said at least one tetrahydropyran nucleoside analog of Formula X:



Bx is a heterocyclic base moiety;

T³ and T⁴ are each, independently, an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound or one of T³ and T⁴ is an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound and the other of T³ and T⁴ is H, a hydroxyl protecting group, a linked conjugate group, or a 5' or 3'-terminal group;

q¹, q², q³, q⁴, q⁵, q⁶, and q⁷ are each, independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl, or substituted C₂-C₆ alkynyl; and

one of R¹ and R² is hydrogen and the other is selected from halogen, substituted or unsubstituted alkoxy, NJ¹J², SJ¹, N₃, OC(=X)J¹, OC(=X)NJ¹J², NJ³C(=X)NJ¹J², and CN, wherein X is O, S, or NJ¹, and each J¹, J², and J³ is, independently, H or C₁-C₆ alkyl.

[0518] In certain embodiments, the modified THP nucleosides of Formula X are provided wherein q^m, qⁿ, q^p, q^r, q^s, q^t, and q^u are each H. In certain embodiments, at least one of q^m, qⁿ, q^p, q^r, q^s, q^t, and q^u is other than H. In certain embodiments, at least one of q^m, qⁿ, 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 R¹ and R² is F. In certain embodiments, R¹ is fluoro and R² is H, R¹ is methoxy and R² is H, and R¹ is methoxyethoxy and R² is H.

[0519] 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-C₁-C₁₀ alkyl, -OCF₃, O-(CH₂)₂-OCH₃, 2’-O(CH₂)₂SCH₃, O-(CH₂)₂-O-N(R^m)(Rⁿ), or O-CH₂-C(=O)-N(R^m)(Rⁿ), where each R^m and Rⁿ is, independently, H or substituted or unsubstituted C₁-C₁₀ alkyl. 2’-modified nucleosides may further comprise other modifications, for example, at other positions of the sugar and/or at the nucleobase.

[0520] As used herein, “2’-F” refers to a sugar comprising a fluoro group at the 2’ position.

[0521] As used herein, “2’-OMe” or “2’-OCH₃” or “2’-O-methyl” each refers to a nucleoside comprising a sugar comprising an -OCH₃ group at the 2’ position of the sugar ring.

[0522] 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).

[0523] 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.

[0524] Methods for the preparations of modified sugars are well known to those skilled in the art.

[0525] 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.

[0526] 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

[0527] 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.

[0528] 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.

[0529] 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.

[0530] 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.

[0531] The disclosure provides pharmaceutical compositions comprising the modified oligonucleotides of the disclosure and a pharmaceutically acceptable carrier, diluent or excipient. In some embodiments, the modified oligonucleotide comprises a sequence of any one SEQ ID NOS: 11-666. In some embodiments, the modified oligonucleotide comprises a sequence of any one SEQ ID NOS: 11-666, and 1, 2, 3, 4, or 5 modifications thereto, as described herein.

[0532] Modified 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.

[0533] The pharmaceutical compositions of the disclosure can optionally comprise therapeutic agents, pharmaceutical agents, carriers, adjuvants, dispersing agents, diluents, and the like.

[0534] 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.

[0535] Pharmaceutical compositions comprising modified oligonucleotides can 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.

[0536] Modified oligonucleotides formulated as prodrugs are contemplated as within the scope of the instant disclosure. 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.

[0537] Pharmaceutical compositions can contain any of the reagents discussed above, and one or more of a pharmaceutically acceptable carrier, a diluent or an excipient.

[0538] A “pharmaceutical composition” is a formulation comprising the modified oligonucleotides described herein, in a form suitable for administration to a subject. In certain embodiments, the pharmaceutical composition is in bulk or in unit dosage form. The unit dosage form is any of a variety of forms, including, for example, a capsule, an IV bag, a tablet, a single use injector, a single pump on an aerosol inhaler or a vial. The quantity of active ingredient (e.g., a formulation of the modified oligonucleotide) in a unit dose of composition is an effective amount and is varied according to the particular treatment involved. One skilled in the art will appreciate that it is sometimes necessary to make routine variations to the dosage depending on the age and condition of the patient. The dosage will also depend on the route of administration. A variety of routes are contemplated, including oral, pulmonary, rectal, parenteral, transdermal, subcutaneous, intravenous, intramuscular, intraperitoneal, inhalational, buccal, sublingual, intrapleural, intrathecal, intranasal, and the like. Dosage forms for the topical or transdermal administration of a of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. In certain embodiments, the modified oligonucleotide is mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that are required.

[0539] “Pharmaceutically acceptable excipient” means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes excipient that is acceptable for veterinary use as well as human pharmaceutical use. A “pharmaceutically acceptable excipient” as used in the specification includes both one and more than one such excipient.

[0540] A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), intraperitoneal (into the body cavity) and transmucosal administration. Solutions or suspensions used for parenteral, intradermal, intraperitoneal or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates,

and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral or subcutaneous preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. These preparations can contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions can include suspending agents and thickening agents. The formulations can be presented in unit/dose or multi-dose containers, for example sealed ampoules, syringes and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use.

[0541] The pharmaceutical composition described herein may be manufactured in a manner that is generally known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

Pharmaceutical compositions may be formulated in a conventional manner using one or more pharmaceutically acceptable carriers comprising excipients and/or auxiliaries that facilitate processing of the active agents into preparations that can be used pharmaceutically. Of course, the appropriate formulation is dependent upon the route of administration chosen.

[0542] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol

and sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0543] Oral compositions generally include an inert diluent or an edible pharmaceutically acceptable carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active age can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the agents in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or agents of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0544] For administration by inhalation, the agents are delivered in the form of an aerosol spray from pressured container or dispenser, which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

[0545] Pharmaceutical compositions can be prepared with pharmaceutically acceptable carriers that will protect the modified oligonucleotides against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art, and the materials can be obtained commercially. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

[0546] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit

containing a predetermined quantity of the modified oligonucleotide calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on the unique characteristics of the active agent and the particular therapeutic effect to be achieved.

[0547] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[0548] Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines, alkali or organic salts of acidic residues such as carboxylic acids, and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids.

[0549] Techniques for formulation and administration of the disclosed compositions of the invention can be found in Remington: the Science and Practice of Pharmacy, 19th edition, Mack Publishing Co., Easton, PA (1995).

[0550] All percentages and ratios used herein, unless otherwise indicated, are by weight. Other features and advantages of the present invention are apparent from the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention. Modified oligonucleotides of the disclosure can 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.

[0551] Modified oligonucleotides 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.

[0552] Modified oligonucleotides of the disclosure can be encapsulated within, or incorporated on the surface of particles. In certain embodiments, the particle is a nanoparticle. Exemplary nanoparticles include liposomes, micelles, polymer-based nanoparticles, lipid-polymer based nanoparticles, and polymeric micelles.

[0553] In certain embodiments, the nanoparticle comprises a liposome. Liposomes are spherical vesicles having at least one lipid bilayer, and in some embodiments, an aqueous core. In some embodiments, the lipid bilayer of the liposome may comprise phospholipids. An exemplary but non-limiting example of a phospholipid is phosphatidylcholine, but the lipid bilayer may comprise additional lipids, such as phosphatidylethanolamine. Liposomes may be multilamellar, i.e. consisting of several lamellar phase lipid bilayers, or unilamellar liposomes with a single lipid bilayer. Liposomes can be made in a particular size range that makes them viable targets for phagocytosis. Liposomes can range in size from 20 nm to 100 nm, 100 nm to 400 nm, 1 μ M and larger, or 200 nm to 3 μ M. Examples of lipidoids and lipid-based formulations are provided in U.S. Published Application 20090023673. In other embodiments, the one or more lipids are one or more cationic lipids. One skilled in the art will recognize which liposomes are appropriate for encapsulation of the modified oligonucleotides described herein.

[0554] In certain embodiments, the nanoparticle comprises a micelle. A micelle is an aggregate of surfactant molecules. An exemplary micelle comprises an aggregate of amphiphilic macromolecules, polymers or copolymers in aqueous solution, wherein the hydrophilic head portions contact the surrounding solvent, while the hydrophobic tail regions are sequestered in the center of the micelle.

[0555] In certain embodiments, the nanoparticle comprises a polymer based nanoparticle. Polymer based nanoparticles comprise one or more polymers, such as a polyester, poly(orthoester), poly(ethylene imine), poly(caprolactone), polyanhydride, poly(acrylic acid), polyglycolide or poly(urethane). In still other embodiments, the one or more polymers comprise poly(lactic acid) (PLA) or poly(lactic-co-glycolic acid) (PLGA). In exemplary embodiments, the one or more polymers comprise polyalkylene glycol such as polyethylene glycol (PEG), or polyalkylene oxide, such as polyethylene oxide (PEO).

[0556] In some embodiments, the nanoparticles or some portion thereof are degradable. In other embodiments, the lipids and/or polymers of the nanoparticles are degradable.

Conjugated Antisense compounds

[0557] 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.

[0558] 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

[0559] 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 commercial 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

[0560] Described herein are methods for treatment of cells with antisense oligonucleotides, which can be modified appropriately for treatment with other antisense compounds.

[0561] Cells may be treated with antisense oligonucleotides when the cells reach approximately 60-80% confluency in culture.

[0562] 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.

[0563] 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.

[0564] Another technique used to introduce antisense oligonucleotides into cultured cells includes electroporation.

[0565] 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.

[0566] 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

[0567] 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

[0568] 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 quantitative 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

[0569] 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.

[0570] 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.

[0571] 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 (Invitrogen, 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.

[0572] 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

[0573] 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.

[0574] 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 (Invitrogen, 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.

[0575] 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

[0576] 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

[0577] 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. Administration also includes intra-ocular and intramuscular routes of administration. 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

[0578] 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, Hepatitis Delta virus (HDV) infection (e.g. in subject co-infected with HBV), 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.

[0579] 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 I-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 studies 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).

[0580] 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.

[0581] 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.

[0582] 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.

[0583] 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

[0584] 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.

[0585] 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.

[0586] 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.

Enumerated Embodiments

[0587] While various specific embodiments have been illustrated and described, it will be appreciated that various changes can be made without departing from the spirit and scope of the disclosure(s). The present disclosure is exemplified by the numbered embodiments set forth below.

Set I:

[0588] Embodiment I-1. A compound comprising a modified oligonucleotide of 20 linked nucleosides consisting of:

- a first gap segment consisting of linked nucleosides;
- a second gap segment consisting of linked nucleosides;
- a separator segment consisting of a nucleoside linked to the first gap segment and the second gap segment;
- a 5' wing segment consisting of linked nucleosides; and
- a 3' wing segment consisting of linked nucleosides;

wherein the first gap segment, separator segment, and second gap segment are positioned

between the 5' wing segment and the 3' wing segment;

wherein the first gap segment is attached to the 5' wing segment, and the second gap segment is

attached to the 3' wing segment

wherein the first gap segment, separator segment, and second gap segment, taken together,

consist of from 8 to 12 linked nucleosides.

[0589] Embodiment I-2. The compound of embodiment I-1, wherein the first gap segment consists of linked nucleosides with 2'-deoxy sugars.

[0590] Embodiment I-3. The compound of embodiment I-1 or I-2, wherein the second gap segment consists of linked nucleosides with 2'-deoxy sugars.

[0591] Embodiment I-4. The compound of a preceding embodiment, wherein the separator segment consists of one nucleoside comprising a 2'-O(CH₂)₂-OCH₃ sugar or a 2'-OCH₃ sugar.

[0592] Embodiment I-5. The compound of a preceding embodiment, wherein at least one of the nucleosides in the 5' wing segment comprises a 2'-O(CH₂)₂-OCH₃ sugar.

[0593] Embodiment I-6. The compound of a preceding embodiment, wherein at least one of the nucleosides in the 3' wing segment comprises a 2'-O(CH₂)₂-OCH₃ sugar.

[0594] Embodiment I-7. The compound of a preceding embodiment, wherein the modified oligonucleotide is at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or 100% complementary to a HBV nucleic acid.

[0595] Embodiment I-8. The compound of a preceding embodiment, wherein the modified oligonucleotide has a nucleobase sequence of SEQ ID NO: 2 (GCAGA GGTGA AGCGA AGTGC).

[0596] Embodiment I-9. The compound of a preceding embodiment, wherein the modified oligonucleotide is a single-stranded modified oligonucleotide.

[0597] Embodiment I-10. The compound of a preceding embodiment, wherein at least one internucleoside linkage is a modified internucleoside linkage.

[0598] Embodiment I-11. The compound of embodiment I-10, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.

[0599] Embodiment I-12. The compound of a preceding embodiment, wherein the modified oligonucleotide comprises at least one modified nucleobase.

[0600] Embodiment I-13. The compound of a preceding embodiment, wherein the modified oligonucleotide comprises one, two, or three modified nucleobase(s).

- [0601] Embodiment I-14. The compound of embodiment I-12 or I-13, wherein the modified nucleobase is a 5-methylcytosine.
- [0602] Embodiment I-15. The compound of embodiment I-1, wherein the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine.
- [0603] Embodiment I-16. The compound a preceding embodiment, wherein each of the nucleosides in the 5' wing segment comprise a 2'-O(CH₂)₂-OCH₃ sugar.
- [0604] Embodiment I-17. The compound of a preceding embodiment, wherein each of the nucleosides in the 3' wing segment comprise a 2'-O(CH₂)₂-OCH₃ sugar.
- [0605] Embodiment I-18. The compound of embodiments 1 to I-15, wherein the 5' wing segment comprises a nucleoside with a 2'-deoxy sugar.
- [0606] Embodiment I-19. The compound of embodiment I-18, wherein the other nucleosides in the 5' wing segment comprise a 2'-O(CH₂)₂-OCH₃ sugar.
- [0607] Embodiment I-20. The compound of embodiment I-1, wherein the nucleoside at position 2 of SEQ ID NO: 2 comprises a 2'-deoxy sugar.
- [0608] Embodiment I-21. The compound of embodiment I-1, wherein the 5' wing segment further comprises two nucleosides each with a 2'-deoxy sugar.
- [0609] Embodiment I-22. The compound of embodiment I-1, wherein the nucleosides at positions 2 and 5 of SEQ ID NO: 2 comprise a 2'-deoxy sugar.
- [0610] Embodiment I-23. The compound of embodiment I-1, wherein the 3' wing segment comprises a nucleoside with a 2'-deoxy sugar.
- [0611] Embodiment I-24. The compound of embodiment I-23, wherein the other nucleosides in the 3' wing segment comprise a 2'-O(CH₂)₂-OCH₃ sugar.
- [0612] Embodiment I-25. The compound of embodiment I-1, wherein the nucleoside at position 16 of SEQ ID NO: 2 comprises a 2'-deoxy sugar.
- [0613] Embodiment I-26. The compound of embodiment I-1, wherein one, two, three, or four of the nucleosides in the 5' wing segment comprise a 4'-CH₂-O-2' sugar.
- [0614] Embodiment I-27. The compound of embodiment I-26, wherein two of the nucleosides in the 5' wing segment comprise a 4'-CH₂-O-2' sugar.
- [0615] Embodiment I-28. The compound of embodiment I-1, wherein the nucleosides at positions 1 and 3 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.
- [0616] Embodiment I-29. The compound of embodiment I-1, wherein one, two, three, or four of the nucleosides in the 3' wing segment comprise a 4'-CH₂-O-2' sugar.

- [0617] Embodiment I-30. The compound of embodiment I-29, wherein two of the nucleosides in the 3' wing segment comprise a 4'-CH₂-O-2' sugar.
- [0618] Embodiment I-31. The compound of embodiment I-1, wherein the nucleosides at positions 17 and 18 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.
- [0619] Embodiment I-32. The compound of embodiment I-1, wherein the nucleosides at positions 16 and 19 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.
- [0620] Embodiment I-33. The compound of embodiment I-1, wherein the nucleosides at positions 17 and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.
- [0621] Embodiment I-34. The compound of embodiment I-29, wherein three of the nucleosides in the 3' wing segment comprise a 4'-CH₂-O-2' sugar.
- [0622] Embodiment I-35. The compound of embodiment I-1, wherein the nucleosides at positions 17, 19, and 20 of SEQ ID NO: 2 comprise a 4'-CH₂-O-2' sugar.
- [0623] Embodiment I-36. The compound of a preceding embodiment, wherein the first gap segment consists of 1, 2, 3, 4, 5, 6, 7, or 8 linked nucleosides.
- [0624] Embodiment I-37. The compound of a preceding embodiment, wherein the first gap segment consists of 4 or 5 linked nucleosides.
- [0625] Embodiment I-38. The compound of a preceding embodiment, wherein the first gap segment consists of 4 linked nucleosides.
- [0626] Embodiment I-39. The compound of a preceding embodiment, wherein the second gap segment consists of 1, 2, 3, 4, 5, 6, 7, or 8 linked nucleosides.
- [0627] Embodiment I-40. The compound of a preceding embodiment, wherein the second gap segment consists of 5 or 6 linked nucleosides.
- [0628] Embodiment I-41. The compound of a preceding embodiment, wherein the second gap segment consists of 5 linked nucleosides.
- [0629] Embodiment I-42. The compound of a preceding embodiment, wherein the separator segment is at position 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 of SEQ ID NO: 2.
- [0630] Embodiment I-43. The compound of a preceding embodiment, wherein the separator segment is at position 9 or 10 or 11 of SEQ ID NO: 2.
- [0631] Embodiment I-44. The compound of a preceding embodiment, wherein the separator segment is at position 10 of SEQ ID NO: 2.
- [0632] Embodiment I-45. The compound of embodiment I-1, wherein
the 5' wing segment consists of 5 linked nucleosides; and

the 3' wing segment consists of 5 linked nucleosides;
the first gap segment consists of 4 linked nucleosides with 2'-deoxy sugars;
the second gap segment consists of 5 linked nucleosides with 2'-deoxy sugars;
the separator segment is at position 10 of SEQ ID NO:2;
the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine
each internucleoside linkage is a phosphorothioate internucleoside linkage.

[0633] Embodiment I-46. The compound of embodiment I-1, wherein
the 5' wing segment consists of 4 linked nucleosides; and
the 3' wing segment consists of 5 linked nucleosides;
the first gap segment consists of 5 linked nucleosides with 2'-deoxy sugars;
the second gap segment consists of 5 linked nucleosides with 2'-deoxy sugars;
the separator segment is at position 10 of SEQ ID NO:2;
the cytosines at positions 2, 13, and 20 of SEQ ID NO: 2 are each 5-methylcytosine;
each internucleoside linkage is a phosphorothioate internucleoside linkage.

[0634] Embodiment I-47. The compound of embodiment I-1, wherein
the 5' wing segment consists of 5 linked nucleosides; and
the 3' wing segment consists of 4 linked nucleosides;
the first gap segment consists of 4 linked nucleosides with 2'-deoxy sugars;
the second gap segment consists of 6 linked nucleosides with 2'-deoxy sugars; and
the separator segment is at position 10 of SEQ ID NO:2;
each internucleoside linkage is a phosphorothioate internucleoside linkage.

[0635] Embodiment I-48. The compound of embodiment I-1, wherein
the 5' wing segment consists of 4 linked nucleosides; and
the 3' wing segment consists of 6 linked nucleosides;
the first gap segment consists of 5 linked nucleosides with 2'-deoxy sugars;
the second gap segment consists of 4 linked nucleosides with 2'-deoxy sugars; and
the separator segment is at position 10 of SEQ ID NO:2;
each internucleoside linkage is a phosphorothioate internucleoside linkage.

[0636] Embodiment I-49. The compound of embodiment I-1, wherein
the 5' wing segment consists of 5 linked nucleosides; and
the 3' wing segment consists of 6 linked nucleosides;
the first gap segment consists of 4 linked nucleosides with 2'-deoxy sugars;

the second gap segment consists of 4 linked nucleosides with 2'-deoxy sugars;
each internucleoside linkage is a phosphorothioate internucleoside linkage.

[0637] Embodiment I-50. The compound of embodiment I-1, wherein the modified oligonucleotide has a sequence which is a member selected from the group consisting of A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, and AX.

[0638] Embodiment I-51. A composition comprising the compound of any of embodiments I-1 to I-50 or salt thereof and at least one of a pharmaceutically acceptable carrier or diluent.

[0639] Embodiment I-52. A method comprising administering to an animal the compound or composition of any of embodiments I-1 to I-51.

[0640] Embodiment I-53. The method of embodiment I-52, wherein the animal is a human.

[0641] Embodiment I-54. The method of embodiment I-52, wherein administering the compound prevents, treats, ameliorates, or slows progression of a HBV-related disease, disorder or condition.

[0642] Embodiment I-55. The method of embodiment I-54, wherein the disease, disorder or condition is liver disease.

[0643] Embodiment I-56. The method of embodiment I-54, 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.

[0644] Embodiment I-57. The method of embodiment I-54, wherein the disease or condition is a hyperproliferative condition.

[0645] Embodiment I-58. The method of embodiment I-57, wherein the hyperproliferative condition is liver cancer.

[0646] Embodiment I-59. A method of reducing HBV antigen levels in an animal comprising administering to said animal the compound or composition of any of embodiments I-1 to I-51.

[0647] Embodiment I-60. The method of embodiment I-59, wherein HBsAg levels are reduced.

[0648] Embodiment I-61. The method of embodiment I-59, wherein HBeAg levels are reduced.

[0649] Embodiment I-62. The method of embodiment I-59, wherein the animal is human.

Set II:

[0650] Embodiment II-1. A compound comprising a modified oligonucleotide, the modified oligonucleotide comprising from 5' to 3':

5' W1 - G1 - S1 - G2 - W2 3' (Formula I)

wherein:

W1 is a 5' wing segment;

W2 is a 3' wing segment;

G1 is a first gap segment;

S1 is a first separator segment;

G2 is a second gap segment;

- is an internucleoside linkage; and

at least one nucleoside of the oligonucleotide is modified.

[0651] Embodiment II-2. The compound of embodiment II-1, wherein the modified oligonucleotide comprises from 5' to 3':

5' W1 - G1 - S1 - G2 - S2 - G3 - W2 3' (Formula II)

wherein:

S2 is a second separator segment; and

G3 is a third gap segment.

[0652] Embodiment II-3. The compound of embodiment II-2, wherein the modified oligonucleotide comprises from 5' to 3':

5' W1 - G1 - S1 - G2 - S2 - G3 - S3 - G4 - W2 3' (Formula III)

wherein:

S3 is a third separator segment; and

G4 is a fourth gap segment.

[0653] Embodiment II-4. The compound of embodiment II-3, wherein the modified oligonucleotide comprises from 5' to 3':

5' W1 - G1 - S1 - G2 - S2 - G3 - S3 - G4 - S4 - G5 - W2 3' (Formula IV)

wherein:

S4 is a fourth separator segment; and

G5 is a fifth gap segment.

[0654] Embodiment II-5. The compound of embodiment II-4, wherein the modified oligonucleotide comprises from 5' to 3':

5' W1 - G1 - S1 - G2 - S2 - G3 - S3 - G4 - S4 - G5 - S5 - G6 - W2 3' (Formula V)

wherein:

S5 is a fifth separator segment; and

G6 is a sixth gap segment.

[0655] Embodiment II-6. The compound of embodiment II-5, wherein the modified oligonucleotide comprises from 5' to 3':



(Formula VI)

wherein:

S6 is a sixth separator segment; and

G7 is a seventh gap segment.

[0656] Embodiment II-7. The compound of any one of embodiments II-1 to II-6, wherein W1 comprises one or more linked deoxynucleosides.

[0657] Embodiment II-8. The compound of any one of embodiments II-1 to II-7, wherein W2 comprises one or more linked deoxynucleosides.

[0658] Embodiment II-9. The compound of any one of embodiments II-1 to II-8, wherein W1 comprises 2 to 25 linked nucleosides.

[0659] Embodiment II-10. The compound of any one of embodiments II-1 to II-9, wherein W2 comprises 2 to 35 linked nucleosides.

[0660] Embodiment II-11. The compound of any one of embodiments II-1 to II-10, wherein G1 comprises 1 to 10 linked deoxynucleosides.

[0661] Embodiment II-12. The compound of any one of embodiments II-1 to II-11, wherein G2 comprises 1 to 10 linked deoxynucleosides.

[0662] Embodiment II-13. The compound of any one of embodiments II-1 to II-12, wherein S1 comprises 1 to 3 linked nucleosides.

[0663] Embodiment II-14. The compound of any one of embodiments II-2 to II-13, wherein G3 comprises 1 to 10 linked deoxynucleosides.

[0664] Embodiment II-15. The compound of any one of embodiments II-2 to II-14, wherein S2 comprises 1 to 3 linked nucleosides.

[0665] Embodiment II-16. The compound of any one of embodiments II-3 to II-15, wherein G4 comprises 1 to 10 linked deoxynucleosides.

[0666] Embodiment II-17. The compound of any one of embodiments II-3 to II-16, wherein S3 comprises 1 or 2 linked nucleosides.

[0667] Embodiment II-18. The compound of any one of embodiments II-4 to II-17, wherein G5 comprises 1 to 10 linked deoxynucleosides.

- [0668] Embodiment II-19. The compound of any one of embodiments II-4 to II-17, wherein S4 comprises 1 to 3 linked nucleosides.
- [0669] Embodiment II-20. The compound of any one of embodiments II-5 to II-19, wherein G6 comprises 1 to 10 linked deoxynucleosides.
- [0670] Embodiment II-21. The compound of any one of embodiments II-5 to II-19, wherein S5 comprises 1 to 3 linked nucleosides.
- [0671] Embodiment II-22. The compound of any one of embodiments II-6 to II-21, wherein G7 comprises 1 to 10 linked deoxynucleosides.
- [0672] Embodiment II-23. The compound of any one of embodiments II-6 to II-22, wherein S6 comprises 1 to 3 linked nucleosides.
- [0673] Embodiment II-24. The compound of any one of embodiments II-1 to II-23, wherein the oligonucleotide is 18-50 nucleobases in length.
- [0674] Embodiment II-25. The compound of any one of embodiments II-1 to II-23, wherein the oligonucleotide is at least 20 nucleobases in length.
- [0675] Embodiment II-26. The compound of any one of embodiments II-1 to II-23, wherein the oligonucleotide is 20 nucleobases in length.
- [0676] Embodiment II-27. The compound of embodiment II-1, wherein the oligonucleotide is 20 linked nucleobases in length.
- [0677] Embodiment II-28. The compound of embodiment II-27, wherein W1 comprises 4 linked nucleosides.
- [0678] Embodiment II-29. The compound of embodiment II-27, wherein W1 comprises 5 linked nucleosides.
- [0679] Embodiment II-30. The compound of any one of embodiments II-27 to II-29, wherein G1 comprises 4 linked deoxynucleosides.
- [0680] Embodiment II-31. The compound of any one of embodiments II-27 to II-29, wherein G1 comprises 5 linked deoxynucleosides.
- [0681] Embodiment II-32. The compound of any one of embodiments II-27 to II-29, wherein G1 comprises 6 linked deoxynucleosides.
- [0682] Embodiment II-33. The compound of any one of embodiments II-27 to II-32, wherein G2 comprises 4 linked deoxynucleosides.
- [0683] Embodiment II-34. The compound of any one of embodiments II-27 to II-32, wherein G2 comprises 5 linked deoxynucleosides.

[0684] Embodiment II-35. The compound of any one of embodiments II-27 to II-32, wherein G2 comprises 6 linked deoxynucleosides.

[0685] Embodiment II-36. The compound of any one of embodiments II-27 to II-33, wherein S1 comprises at least one linked nucleoside.

[0686] Embodiment II-37. The compound of any one of embodiments II-27 to II-35, wherein W2 comprises 5 linked nucleosides.

[0687] Embodiment II-38. The compound of any one of embodiments II-27 to II-35, wherein W2 comprises 6 linked nucleosides.

[0688] Embodiment II-39. The compound of any one of embodiments II-27 to II-38, wherein the S1 is at position 10 of the oligonucleotide.

[0689] Embodiment II-40. The compound of embodiment II-39, wherein W1 comprises 4 linked nucleosides, G1 comprises 5 linked deoxynucleosides, S1 comprises 1 linked nucleoside, G2 comprises 5 linked deoxynucleosides, and W2 comprises 5 linked nucleosides.

[0690] Embodiment II-41. The compound of embodiment II-1, wherein W1 comprises 4-6 linked nucleosides.

[0691] Embodiment II-42. The compound of embodiment II-41, wherein W2 comprises 4-6 linked nucleosides.

[0692] Embodiment II-43. The compound of any one of embodiments II-41 to II-42, wherein G1 comprises 1-6 linked deoxynucleosides.

[0693] Embodiment II-44. The compound of any one of embodiments II-41 to II-43, wherein G2 comprises 1-6 linked deoxynucleosides.

[0694] Embodiment II-45. The compound of any one of embodiments II-41 to II-44, wherein G3 comprises 1-6 linked deoxynucleosides.

Embodiment II-46. The compound of any one of embodiments II-41 to II-45, wherein S1 comprises least one linked nucleoside.

[0695] Embodiment II-47. The compound of any one of embodiments II-41 to II-46, wherein the length of the G1-S1-G2 is between 8 and 12 nucleobases in length.

[0696] Embodiment II-48. The compound of any one of embodiments II-1 to II-47, wherein any one or more of G1, G2, G3, G4, G5, G6, and G7 comprises a nucleoside comprising a modification to a 2'-deoxynucleoside.

[0697] Embodiment II-49. The compound of embodiment II-48, wherein any one or more of G1, G2, G3, G4, G5, G6, and G7 comprises a nucleoside comprising a 2'-deoxy 5-methylcytidine sugar modification.

[0698] Embodiment II-50. The compound of any one of embodiments II-1 to II-49, wherein S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-O-methoxyethyl sugar modification.

[0699] Embodiment II-51. The compound of any one of embodiments II-1 to II-49, wherein S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 5-methylcytidine.

[0700] Embodiment II-52. The compound of any one of embodiments II-1 to II-49, wherein S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-O-methyl sugar modification.

[0701] Embodiment II-53. The compound of any one of embodiments II-1 to II-52, wherein S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-OH sugar modification.

[0702] Embodiment II-54. The compound of any one of embodiments II-1 to II-53, wherein S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-fluoro sugar modification.

[0703] Embodiment II-55. The compound of any one of embodiments II-1 to II-54, wherein S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-fluoro-arabinonucleic acid (2'-fluoro-ANA) sugar modification.

[0704] Embodiment II-56. The compound of any one of embodiments II-1 to II-55, wherein S1, S2, S3, S4, S5, and/or S6 comprises a glycol nucleic acid (GNA).

[0705] Embodiment II-57. The compound of any one of embodiments II-1 to II-56, wherein S1, S2, S3, S4, S5, and/or S6 comprises a LNA.

[0706] Embodiment II-58. The compound of any one of embodiments II-1 to II-57, wherein W1 comprises a nucleoside comprising a 2'-deoxy sugar modification.

[0707] Embodiment II-59. The compound of embodiment II-58, wherein the 2'-deoxy sugar modification is at positions 2 and/or 5 of a sequence corresponding to SEQ ID NO: 2.

[0708] Embodiment II-60. The compound of any one of embodiments II-1 to II-59, wherein W1 comprises a nucleoside comprising a 2'-O-methoxyethyl sugar modification.

[0709] Embodiment II-61. The compound of embodiment II-60, wherein the 2'-O-methoxyethyl sugar modification is at position 1, 2, 3, 4 and/or 5 of a sequence corresponding to SEQ ID NO: 2.

[0710] Embodiment II-62. The compound of any one of embodiments II-1 to II-61, wherein W1 comprises a 2'-O-methoxyethyl 5-methylcytidine at position 2 of a sequence corresponding to SEQ ID NO: 2.

[0711] Embodiment II-63. The compound of any one of embodiments II-1 to II-62, wherein W1 comprises a nucleoside comprising a 2'-O-methyl sugar modification.

[0712] Embodiment II-64. The compound of embodiment II-63, wherein the 2'-O-methyl sugar modification is at positions 1, 2, 3, 4 and/or 5 of a sequence corresponding to SEQ ID NO: 2.

[0713] Embodiment II-65. The compound of embodiment II-63, wherein W1 comprises a 2'-O-methyl 5-methylcytidine at position 2 of a sequence corresponding to SEQ ID NO: 2.

[0714] Embodiment II-66. The compound of any one of embodiments II-1 to II-65, wherein W1 comprises a nucleoside comprising a 2'-fluoro sugar modification.

[0715] Embodiment II-67. The compound of any one of embodiments II-1 to II-66, wherein W1 comprises a nucleoside comprising a 2'-fluoro-arabinonucleic acid (2'-fluoro-ANA) modification.

[0716] Embodiment II-68. The compound of any one of embodiments II-1 to II-67, wherein W1 comprises a glycol nucleic acid (GNA).

[0717] Embodiment II-69. The compound of any one of embodiments II-1 to II-67, wherein the GNA is at position 2 of a sequence corresponding to SEQ ID NO: 2.

[0718] Embodiment II-70. The compound of any one of embodiments II-1 to II-69, wherein W1 comprises a modified nucleoside and wherein the modified nucleoside is a locked nucleic acid (LNA).

[0719] Embodiment II-71. The compound of embodiment II-70, wherein the LNA is at positions 1 and/or 3 of a sequence corresponding to SEQ ID NO: 2.

[0720] Embodiment II-72. The compound of any one of embodiments II-1 to II-71, wherein W2 comprises a nucleoside comprising a 2'-deoxy sugar modification.

[0721] Embodiment II-73. The compound of embodiment II-72, wherein the 2'-deoxy sugar modification is at positions 15 and/or 16 of a sequence corresponding to SEQ ID NO: 2.

[0722] Embodiment II-74. The compound of any one of embodiments II-1 to II-73, wherein W2 comprises a nucleoside comprising a 2'-O-methoxyethyl sugar modification.

[0723] Embodiment II-75. The compound of embodiment II-74, wherein the 2'-O-methoxyethyl sugar modification is at positions 15, 16, 17, 18, 19 and/or 20 of a sequence corresponding to SEQ ID NO: 2.

[0724] Embodiment II-76. The compound of embodiment II-74, wherein W2 comprises a 2'-O-methoxyethyl 5-methylcytidine at position 20 of a sequence corresponding to SEQ ID NO: 2.

[0725] Embodiment II-77. The compound of any one of embodiments II-1 to II-76, wherein W2 comprises a nucleoside comprising a 2'-O-methyl sugar modification.

[0726] Embodiment II-78. The compound of embodiment II-77, wherein the 2'-O-methyl sugar modification is at positions 15, 16, 17, 18, 19 and/or 20 of a sequence corresponding to SEQ ID NO: 2.

[0727] Embodiment II-79. The compound of embodiment II-77, wherein W2 comprises a 2'-O-methyl 5-methylcytidine at position 20 of a sequence corresponding to SEQ ID NO: 2.

[0728] Embodiment II-80. The compound of any one of embodiments II-1 to II-79, wherein W2 comprises a nucleoside comprising a 2'-fluoro sugar modification.

[0729] Embodiment II-81. The compound of any one of embodiments II-1 to II-80, wherein W2 comprises a nucleoside comprising a 2'-fluoro-arabinonucleic acid (2'-fluoro-ANA) modification.

[0730] Embodiment II-82. The compound of any one of embodiments II-1 to II-81, wherein W2 comprises a modified nucleoside and wherein the modified nucleoside is a glycol nucleic acid (GNA).

[0731] Embodiment II-83. The compound of any one of embodiments II-1 to II-82, wherein W2 comprises a modified nucleoside and wherein the modified nucleoside is a locked nucleic acid (LNA).

[0732] Embodiment II-84. The compound of embodiment II-83, wherein the LNA is at positions 16, 17, 18, 19 and/or 20 of a sequence corresponding to SEQ ID NO: 2.

[0733] Embodiment II-85. The compound of any one of embodiments II-1 to II-84, wherein at least one internucleoside linkage is a phosphodiester linkage.

[0734] Embodiment II-86. The compound of any one of embodiments II-1 to II-85, wherein at least one internucleoside linkage is a modified internucleoside linkage.

[0735] Embodiment II-87. The compound of embodiment II-86, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

[0736] Embodiment II-88. The compound of any one of embodiments II-1 to II-86, wherein the oligonucleotide is complementary to a portion of the polynucleotide sequence of a HBV genome.

[0737] Embodiment II-89. The compound of embodiment II-88, wherein the HBV genome is the genome of HBV GT-A, GT-B, GT-C, GT-D, GT-E, GT-F, GT-G, or GT-H.

[0738] Embodiment II-90. The compound of embodiment II-88, wherein the oligonucleotide is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% complementary to a portion of the polynucleotide sequence of any one of SEQ ID NOS: SEQ ID NO 3, and 667-674.

[0739] Embodiment II-91. The compound of embodiment II-88, wherein the oligonucleotide is complementary to the polynucleotide sequence of any one of SEQ ID NOS: 1, 4, and 675-838.

[0740] Embodiment II-92. The compound of any one of embodiments II-1 to II-91, wherein the oligonucleotide comprises a nucleobase sequence of a sequence corresponding to SEQ ID NO: 2 or a nucleobase sequence of.

[0741] Embodiment II-93. A compound comprising the modified oligonucleotide of any one of SEQ ID NOS: 11-666, or a oligonucleotide comprising at least 1, 2, 3, 4, or 5 further modifications thereto.

[0742] Embodiment II-94. A compound comprising the modified oligonucleotide of any one of SEQ ID NOS: 11-666, or a oligonucleotide having at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% sequence identity thereto.

[0743] Embodiment II-95. A pharmaceutical composition comprising the compound of any one of embodiments II-1 to II-94, or salt thereof and at least one pharmaceutically acceptable carrier or diluent.

[0744] Embodiment II-96. A formulation comprising about 100 mg/mL, 150 mg/mL, or 200 mg/mL of a compound comprising the modified oligonucleotide sequence of SEQ ID NO: 456, or a oligonucleotide comprising at least 1, 2, 3, 4, or 5 further modifications thereto.

[0745] Embodiment II-97. A method of reducing HBV antigen levels in a subject comprising administering to the subject the compound of any one of embodiments II-1 to II-94, the composition of embodiment II-95 or the formulation of embodiment II-96.

[0746] Embodiment II-98. The method of embodiment II-97, wherein the HBV antigen is HBsAg or HBeAg.

[0747] Embodiment II-99. The method of embodiment II-97, wherein HBeAg levels are reduced.

[0748] Embodiment II-100. The method of embodiment II-97, wherein HBsAg levels are reduced.

[0749] Embodiment II-101. The method of embodiment II-97, wherein, wherein the HBsAg levels are reduced by at least about 1.25 fold relative to a subject administered with a composition comprising the modified oligonucleotide sequence of SEQ ID NO: 10.

[0750] Embodiment II-102. The method of embodiment II-97, wherein the HBsAg levels are reduced by about 2 fold to about 10 fold relative to a subject administered with a composition comprising the modified oligonucleotide sequence of SEQ ID NO: 10.

[0751] Embodiment II-103. A method of reducing liver toxicity and/or persistence in a subject having HBV comprising administering to a subject the compound of any one of embodiments II-1 to 94, the composition of embodiment II-95 or the formulation of embodiment II-96.

[0752] Embodiment II-104. The method of embodiment II-103, wherein ALT, ALP, and/or AST levels are reduced, optionally by at least about 1.25 fold relative to a subject administered with a composition comprising the modified oligonucleotide sequence of SEQ ID NO: 10.

[0753] Embodiment II-105. A method of preventing, treating, ameliorating or slowing the progression of a HBV-related disease, disorder or condition comprising administering to a subject the compound of any one of embodiments II-1 to II-94, the composition of embodiment II-95 or the formulation of embodiment II-96.

[0754] Embodiment II-106. The method of any one of embodiments II-97 to II-105, wherein the subject is administered with at least one, at least 2, at least 3, at least 4 or at least 5 doses of the compound, the composition or the formulation.

[0755] Embodiment II-107. The method of any one of embodiments II-97 to II-106, wherein the subject is dosed on Day 1, Day 4 and Day 8.

[0756] Embodiment II-108. The method of embodiment II-107, wherein the subject is dosed once every week after Day 8.

[0757] Embodiment II-109. The method of any one of embodiments II-97 to II-108, wherein the subject is dosed on at least Day 1, Day 4 and Day 8, Day 15 and Day 22.

[0758] Embodiment II-110. The method of any one of embodiments II-107 to II-109, wherein the amount of each dose is substantially the same amount.

[0759] Embodiment II-111. The method of any one of embodiments II-97 to II-110, wherein the dose is about 30 mg to about 600 mg.

[0760] Embodiment II-112. The method of any one of embodiments II-97 to II-110, wherein the dose is about 100 mg to about 500 mg.

[0761] Embodiment II-113. The method of any one of embodiments II-97 to II-110, wherein the dose is about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 450 mg, or about 500 mg.

[0762] Embodiment II-114. The method of any one of embodiments II-97 to II-113, wherein the administering to a subject is parenteral.

[0763] Embodiment II-115. The method of any one of embodiments II-97 to II-113, wherein the administering to a subject is subcutaneous.

[0764] Embodiment II-116. The method of any one of embodiments II-97 to II-113, wherein the administering to a subject is transdermal.

[0765] Embodiment II-117. The method of any one of embodiments II-97 to II-113, wherein the administering to a subject is intraocular.

[0766] Embodiment II-118. The method of any one of embodiments II-97 to II-113, wherein the administering to a subject is intramuscular.

[0767] Embodiment II-119. The method of any one of embodiments II-97 to II-113, wherein the administering to a subject is intravenous.

[0768] Embodiment II-120. The method of any one of embodiments II-97 to II-115, wherein the method further comprises administering at least a second agent to the subject.

[0769] Embodiment II-121. The method of any one of embodiments II-97 to II-120, wherein the disease, disorder or condition is a liver disease, disorder or condition.

[0770] Embodiment II-122. The method of embodiment II-121, 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 infection, HBV viremia, or liver disease-related transplantation.

[0771] Embodiment II-123. The method of embodiment II-121, wherein the disease or condition is a hyperproliferative condition, optionally liver cancer.

[0772] Embodiment II-124. The method of any one of embodiments II-97 to II-120, wherein the disease, disorder or condition is a hepatitis delta virus infection (HDV), wherein the subject is co-infected with HBV.

[0773] Embodiment II-125. The method of any one of embodiments II-97 to II-124, wherein the subject is human.

EXAMPLES

Non-limiting disclosure and incorporation by reference

[0774] 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: Modified Oligonucleotide Sequences and Methods of Making

[0775] Structures of the modified oligonucleotides of the invention (alternatively known as enhanced AntiSense Oligonucleotides [eASO]) are shown in Tables 1.1 and Table 1.2 (FIGS. 1-2). Table 1.1 shows a legend describing the individual modifications at each position of the modified oligonucleotides. Table 1.2 shows the sequence of each modified oligonucleotides, aligned by position number.

[0776] Synthesis: Using solid-phase synthesis to produce an oligonucleotide. This means that oligonucleotides will be tethered to a solid surface when they are being made. In other words, within the solid-phase synthesis, an oligonucleotide being assembled is covalently bound, via its 3'-terminal hydroxyl group, to solid support material and remains attached to it over the entire course of the chain assembly. This synthesis process begins with the 3'-most nucleotide and proceeds through a series of cycles composed of four steps that are repeated until the 5'-most nucleotide is attached. These four steps are deprotection (A), condensation (B), capping (C), and oxidation (D) are shown in FIG. 3 and stated below:

Step 1: Remove the DMTr attached to the 5'-OH group of the first base on the CPG monomer, and prepare to attach the next new base;

Step 2: Activate the new base monomer and prepare to react with the first base;

Step 3: The second base has a coupling reaction with the first base;

Step 4: Cap the 5'-OH of the first base that has not reacted so that it will no longer participate in the reaction;

Step 5: Oxidation of nucleoside phosphites to more stable nucleoside phosphates;

Step 6: Repeat the cycle of Step1~Step5 until the required Oligo DNA sequence is synthesized.

[0777] Aminolysis--The synthesized oligonucleotide is chemically cleaved from the solid carrier (CPG), and the protective group is removed by aminolysis.

[0778] Purification--The modified primer purification method is as follows:

- (1) PAGE purification: use denaturing polyacrylamide gel electrophoresis to separate the primer DNA then the target DNA is recovered from the gel. The purity of the purified DNA is greater than 90%.
- (2) HPLC purification: use the principle of high performance liquid chromatography to purify the primer DNA to a purity greater than 90%.

Example 2: Antiviral effect of modified oligonucleotides in HepG2.2.15 cells

[0779] The antiviral effect of modified oligonucleotides was evaluated by measuring the levels of hepatitis B virus (HBV) surface antigen (HBsAg) and HBV e antigen (HBeAg) in HepG2.2.15 cells. The modified oligonucleotides used in this study included **AUS1233/AUS1138, AUS1463, AUS1476/AUS1493, AUS1494, AUS1466/AUS1495.**

[0780] HepG2.2.15 cells that can stably express HBsAg and HBeAg were purchased from China Center for Type Culture Collection (CCTCC No. GDC0141). The modified oligonucleotide solutions were prepared by dissolving each compound in saline or RNase-free double distilled H₂O (dd H₂O) to make a target solution at 50 μ M.

[0781] The modified oligonucleotides worked with HepG2.2.15 cells by reverse transfection. First, a cell suspension with a concentration of 3.5×10^5 cells in every 100 μ L suspension was prepared. The cell culture plate was covered with 30-50 μ L Collagen I, Rat Tail (3 mg/mL) (Gibco) in every well. Excess liquid was removed one hour later. P3000 (Invitrogen) and Lipofectamine® 3000 Reagent (Invitrogen) were then diluted with Opti-MEM™ I Reduced Serum Medium (Gibco), mixed with the diluted compound, and then allowed to stand at room temperature for 10 minutes. 10 μ L of transfection reagent mixture of P3000, Lipo3000 and Opti-MEM and 100 μ L of cell suspension were added to each well. The mixture was pipetted 4 or 5 times and the plate was placed in a 37°C incubator.

[0782] HBsAg levels in the cell supernatant were measured using HBsAg ELISA kits (Lot number: 20201116) were purchased from Autobio Diagnostics (China). HBsAg level was measured according to manufacturer's instructions.

[0783] Cell proliferation - toxicity was measured by CCK8 kits. Briefly, the cell supernatant was removed carefully 48 hours after modified oligonucleotide treatment. 100 μ L CCK-8 solution according to manufacturer's instructions were added to each well. The plate was placed in a 37°C incubator for 60min or 90min and the absorbance value in each sample was measured by an enzyme-label analyzer (Perlong DNM-9602).

[0784] Data for the compounds are provided. The EC50 was $1.45 \text{ (nM)} \pm 0.21$ for AUS1233/AUS1138, 0.32 ± 0.11 for AUS1463, 0.28 ± 0.09 for AUS1494, 0.24 ± 0.07 for AUS1476/AUS1493, 0.54 ± 0.21 for AUS1466/AUS1495, the CC50 (nM) was 61.98 ± 9.32 for AUS1233, AUS1138, 57.61 ± 6.14 for AUS1463, 72.91 ± 9.95 for AUS1494, 74.73 ± 8.67 for AUS1476/AUS1493, 72.36 ± 11.77 for AUS1466/AUS1495. AUS1463, AUS1494, AUS1476/AUS1493, AUS1466/AUS1495 reduce HBV antiviral activity EC50 (nM) value is 2.5~4 fold lower than AUS1233/AUS1138 and have similar cellular toxicity value CC50 (nM) as AUS1233/AUS1138.

Compounds	AUS1233/AUS1138	AUS1463	AUS1494	AUS1476/ AUS1493	AUS1466/ AUS1495
EC50 (nM)	1.45 ± 0.21	0.32 ± 0.11	0.28 ± 0.09	0.24 ± 0.07	0.54 ± 0.21
CC50 (nM)	61.98 ± 9.32	57.61 ± 6.14	72.91 ± 9.95	74.73 ± 8.67	72.36 ± 11.77

Example 3: Antiviral effect of modified oligonucleotides in genotype C HBV transgenic mice

[0785] This study evaluated the effect of modified oligonucleotides on the serum levels of HBsAg in genotype C HBV (HBV-GTC) transgenic mice. The modified oligonucleotides used in this study included AUS1233/AUS1138, AUS1463, AUS1466/AUS1495, AUS1494, AUS1476/AUS1493, and AUS1441. Each compound was dosed once at 30 mg/kg by subcutaneous injection (SC) to the mice. Normal saline and AUS1233, AUS1138 were administered as negative control and positive control, respectively.

[0786] This study evaluated the effect of modified oligonucleotides on the serum levels of HBsAg in genotype C HBV (HBV-GTC) transgenic mice. The modified oligonucleotides used in this study included AUS1233/AUS1138, AUS1463, AUS1466/AUS1495, AUS1494, AUS1476/AUS1493, and AUS1441. Each compound was dosed once at 30 mg/kg by subcutaneous injection (SC) to the mice. Normal saline and AUS1233, AUS1138 were administered as negative control and positive control, respectively.

[0787] HBV transgenic mice, prepared from male C57BL/6 mice of 5-7 weeks old with 1.0-fold length of HBV C1 subtype genome, stably express HBsAg in liver. The methods and purposes of animal experiments are in accordance with the ethical standards and international practices.

[0788] AUS1233/AUS1138 was used as a reference compound in this study. HBsAg ELISA kits were purchased from Autobio Diagnostics. ELISA was performed using a

chemiluminescence immunoassay analyzer (Instrument model: LUMO) at Autobio.

Centrifugation of blood was performed with a Preparative Ultracentrifuge by Thermo Fisher Scientific (USA).

[0789] The dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 10 mg/ml, which was diluted with saline again at a target concentration of 3 mg/ml.

[0790] Animal Dosing--The overall study design is shown in the FIG. 4. Male HBV transgenic mice were randomly divided into groups of 4 mice. Each group was dosed with one compound by subcutaneous injection at 30 mg/kg and a dose volume of 10 ml/kg of body weight or an equal volume of normal saline as control.

[0791] Approximately 80 μ l of blood sample was taken from a thigh vein on 0 (before dosing), 3, 7, 10, 14, 21 and 28 days after dosing. Blood samples were placed in an incubator at 37°C for approximately 30 minutes, and then centrifuged in a precooled (0-4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further analysis.

[0792] Serum HBsAg levels in the serum samples were measured using HBsAg ELISA kits (Lot number: 20201116) were purchased from Autobio Diagnostics. Briefly, 50 μ l of 10-fold diluted mouse serum or standards was added to a 96-well plate, HBsAg level was measured according to manufacturer's instructions.

[0793] The HBsAg levels were measured in the serum samples by ELISA. The maximum log₁₀ serum HBsAg reduction was -0.494 ± 0.173 for AUS1233, AUS1138, -1.66 ± 0.52 for AUS1463, -1.43 ± 0.12 for AUS1466/AUS1495, -1.22 ± 0.39 for AUS1494, -1.47 ± 0.26 for AUS1476/AUS1493, and -1.48 ± 0.27 for AUS1441. The body weight change was comparable with the saline group for all treatment groups. The results showed that the antiviral effects of the present modified oligonucleotides were much better than the AUS1233, AUS1138.

Example 4: Antiviral effect of modified oligonucleotides in genotyped HDI-HBV carrier mice

[0794] This study evaluated the effect of modified oligonucleotides on the serum levels of HBsAg in HDI HBV mice. HBV HDI mice model based on the hydrodynamic injection of PreS2/S plasmid into male C57BL/6 mice. The modified oligonucleotides used in this study included AUS1233/AUS1138, AUS1468, AUS1469, AUS1470, AUS1471, AUS1472, AUS1458, AUS1459, AUS1426, AUS1427/AUS1461, AUS1462, AUS1463, AUS1464,

AUS1465, AUS1466/AUS1495, and AUS1467. Each compound was dosed once at 40 mg/kg by SC. Normal saline and **AUS1233, AUS1138** were administered as negative control and positive control, respectively. The HBsAg levels and serum alanine aminotransferase (ALT) levels were measured in the serum samples by ELISA.

[0795] This study evaluated the effect of modified oligonucleotides on the serum levels of HBsAg in HDI HBV mice. HBV HDI mice model based on the hydrodynamic injection of PreS2/S plasmid into male C57BL/6 mice. The modified oligonucleotides used in this study included **AUS1233/AUS1138, AUS1468, AUS1469, AUS1470, AUS1471, AUS1472, AUS1458, AUS1459, AUS1426, AUS1427/AUS1461, AUS1462, AUS1463, AUS1464, AUS1465, AUS1466/AUS1495, and AUS1467.** Each compound was dosed once at 40 mg/kg by SC. Normal saline and **AUS1233, AUS1138** were administered as negative control and positive control, respectively. The HBsAg levels and serum alanine aminotransferase (ALT) levels were measured in the serum samples by ELISA.

[0796] HBsAg ELISA kits were purchased from Autobio Diagnostics (China). ALT ELISA kits were purchased from Elabscience Biotechnology Co., Ltd (China). Normal saline was purchased from Beijing Sanyao Science & Technology Development (China). ELISA was performed using Chemiluminescence immunoassay analyzer of Autobio. Centrifugation of blood was performed with a Preparative Ultracentrifuge of Thermo Fisher Scientific (USA).

[0797] The dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 10 mg/ml, which was diluted with saline again at a target concentration of 4 mg/ml.

Animal Dosing--The overall study design is shown in FIG. 5. Male HDI-HBV carrier mice were randomly divided into every group. Each group was dosed with one compound at 40 mg/kg and a dose volume of 10 ml/kg of body weight or an equal volume of normal saline as control. HBsAg-positive mice were dosed 28 days after HDI modeling at the animal laboratory of Eyoung via SC.

[0798] Approximately 80 µl of blood sample was taken from a thigh vein on 0 (before dosing), 3, 7, 14 and 21 days after dosing. Blood samples were placed in an incubator at 37°C for approximately 30 minutes, and then centrifuged in a precooled (0-4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further analysis.

[0799] Serum HBsAg levels in the serum samples were measured using HBsAg ELISA kits (Lot number: 20201116) were purchased from Autobio Diagnostics (China). Briefly, 50 μ l of 25-fold diluted mouse serum or standards was added to a 96-well plate, HBsAg level was measured according to manufacturer's instructions.

[0800] Serum ALT levels were measured using ALT ELISA kits (Number: E-BC-K235-M) were purchased from Elabscience Biotechnology Co., Ltd (China). Briefly, 50 μ l of 2-fold diluted mouse serum or standards was added to a 96-well plate, HBsAg level was measured according to manufacturer's instructions.

[0801] The maximum log₁₀ serum HBsAg reduction was 1.03 ± 0.23 for AUS1233/AUS1138, and was 2.29 ± 0.25 for AUS1468, 2.26 ± 0.46 for AUS1469, 2.30 ± 0.39 for AUS1470, 2.59 ± 0.39 for AUS1471, 2.59 ± 0.26 for AUS1472, 2.82 ± 0.72 for AUS1458, 2.46 ± 0.52 for AUS1459, 2.57 ± 0.42 for AUS1426, 2.51 ± 0.37 for AUS1427/AUS1461, 2.12 ± 0.21 for AUS1462, 2.03 ± 0.14 for AUS1463, 2.77 ± 1.22 for AUS1464, 2.33 ± 1.10 for AUS1465, 1.90 ± 0.10 for AUS1466/AUS1495 and 1.96 ± 0.35 for AUS1467. The serum ALT levels were < 42 IU/L in all samples for all compounds, indicated the liver were not damaged. The body weight change was comparable with the saline group for all treatment groups.

Example 5: Acute toxic effect of modified oligonucleotides in C57BL/6 mice

[0802] This study evaluated the acute toxic effect of modified oligonucleotides on the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in C57BL/6 mice. The modified oligonucleotides used in this study included AUS1233/AUS1138, AUS1472, AUS1427/AUS1461, AUS1463. Each compound was dosed three times on Day 0, 2, and 4 at 60 mg/kg by subcutaneous injection (SC). Normal saline was used as a negative control. The blood routine was measured in the whole blood samples by HITACHI AUTOMATIC ANALYZER 3100. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured in the serum samples by enzyme-linked immune-sorbent assays (ELISA).

[0803] Male C57BL/6 mice were purchased from Shanghai SLAC Laboratory Animal CO., Ltd (China). The mice were maintained under specific pathogen-free condition in the BSL-2+ animal facility of Hagnzhou Eyoung Biotechnology Co., Ltd (China). The methods and purposes of animal experiments are in accordance with the ethical standards and international practices.

[0804] ALT and AST ELISA kits were purchased from Elabscience Biotechnology Co., Ltd (China). Normal saline was purchased from Beijing Sanyao Science & Technology Development (China). ELISA was performed using Enzyme Immunoassay Analyzer of

Autobio. Centrifugation of blood was performed with a Preparative Ultracentrifuge of Thermo Fisher Scientific (USA).

[0805] The dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 10 mg/ml, which was diluted with saline again at a target concentration of 6 mg/ml.

[0806] The overall study design is shown in FIG. 6. Male C57BL/6 mice were randomly divided into every group. Each compound was dosed three times on Day 0, 2, and 4 at 60 mg/kg by SC and a dose volume of 10 μ l/g of body weight or an equal volume of normal saline as control.

[0807] Approximately 80 μ l of blood sample was taken from a thigh vein on 5, 7, 10, 14, 17 and 21 days after dosing. Blood samples were placed in an incubator at 37°C for approximately 30 minutes, and then centrifuged in a precooled (0–4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further analysis. Approximately 50 μ l of blood sample was taken from a thigh vein on Day 17 after dosing. Blood samples were anticoagulated by EDTA-2Na anticoagulant and diluted. The obtained anticoagulant blood dilution samples were stored in a 4°C refrigerator for blood routine analysis.

[0808] Serum ALT levels were measured using ALT ELISA kits (Number: E-BC-K235-M) were purchased from Elabscience Biotechnology Co., Ltd (China). Briefly, 50 μ l of 2-fold diluted mouse serum or standards was added to a 96-well plate, HBsAg level was measured according to manufacturer's instructions.

[0809] The maximum serum ALT was 53.3 (IU/L) $\pm$ 16.0 for AUS1233/AUS1138, 29.3 $\pm$ 8.2 for AUS1472, 44.9 $\pm$ 22.0 for AUS1427/AUS1461, 16.1 $\pm$ 4.8 for AUS1463. The maximum serum AST (IU/L) was 27.4 $\pm$ 7.9 for AUS1233/AUS1138, 18.3 $\pm$ 5.5 for AUS1472, 19.0 $\pm$ 5.4 for AUS1427/AUS1461, 14.3 $\pm$ 3.4 for AUS1463. The ratio of liver and spleen to body weight in all treatment groups was uncomparable with the saline group. The body weight change was comparable with the saline group for all treatment groups. The results of maximum serum ALT and AST show that the modified oligonucleotides have better drug safety than the AUS1233/AUS1138.

Example 6: Antiviral effect of modified oligonucleotides in pAAV-1.2HBV-GTA HDI-HBV carrier mice

[0810] This study evaluated the effect of modified oligonucleotides on the serum levels of HBsAg in HBV transgenic mice. The modified oligonucleotides used in this study included AUS1233/AUS1138, AUS1444, AUS1458, AUS1459, AUS1460, AUS1427/AUS1461, AUS1462, AUS1463, AUS1464, AUS1465, AUS1463, AUS1467, AUS1468, AUS1470, AUS1471, AUS1472, AUS1473, AUS1474, AUS1475, AUS1476/AUS1493, AUS1478, AUS1479, AUS1489, AUS1490, AUS1443, and AUS1322. Each compound was dosed once at 40 mg/kg by SC. Normal saline was administered as negative control. The HBsAg levels were measured in the serum samples by ELISA.

[0811] Male C57BL/6 mice were purchased from Shanghai SLAC Laboratory Animal CO., Ltd (China). The HDI-HBV mouse model was constructed by hydrodynamic-injection a pAAV-1.2HBV-GTA plasmid containing 1.2-fold length of HBV sequence. HBsAg-positive mice were used Day 18 after the injection. The mice were maintained under specific pathogen-free condition in BSL-2+ animal facility of Hagnzhou Eyoung biotechnology Co., Ltd (China). The methods and purposes of animal experiments are in accordance with the ethical standards and international practices.

[0812] AUS1233/AUS1138 was used as a reference compound in this study. HBsAg ELISA kits were purchased from Autobio Diagnostics (China). Normal saline was purchased from Beijing Sanyao Science & Technology Development (China). ELISA was performed using Chemiluminescence immunoassay analyzer (Instrument model: LUMO) of Autobio. Centrifugation of blood was performed with a Preparative Ultracentrifuge of Thermo Fisher Scientific (USA).

[0813] The dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 10 mg/ml, which was diluted with saline again at a target concentration of 4 mg/ml.

[0814] Animal Dosing: The overall study design is shown in FIG. 7. Male HDI-HBV carrier mice were randomly divided into each group. Each group was dosed with one compound at 40 mg/kg and a dose volume of 10 ml/kg of body weight or an equal volume of normal saline as control. HBsAg-positive mice were dosed 18 days after HDI modeling at the animal laboratory of Eyoung via SC.

[0815] Approximately 80 μ l of blood sample was taken from a thigh vein on 0 (before dosing), 3, 7, 10, 14 and 21 days after dosing. Blood samples were placed in an incubator at 37°C for approximately 30 minutes, and then centrifuged in a precooled (0–4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further analysis.

[0816] Serum HBsAg levels in the serum samples were measured using HBsAg ELISA kits (Lot number: 20201116) were purchased from Autobio Diagnostics (China). Briefly, 50 μ l of 25-fold diluted mouse serum or standards was added to a 96-well plate, HBsAg level was measured according to manufacturer's instructions.

[0817] The maximum log₁₀ serum HBsAg reduction was -0.774 ± 0.111 for AUS1233/AUS1138, -3.61 ± 1.65 for AUS1444, -2.72 ± 1.24 for AUS1458, -1.27 ± 0.21 for AUS1459, -2.06 ± 1.78 for AUS1460, -1.21 ± 0.35 for AUS1427/AUS1461, -2.09 ± 2.23 for AUS1462, -2.06 ± 0.05 for AUS1463, -1.70 ± 1.39 for AUS1464, -2.09 ± 1.70 for AUS1465, -1.38 ± 0.53 for AUS1463, -2.80 ± 2.11 for AUS1467, -0.844 ± 0.262 for AUS1468, -0.910 ± 0.259 for AUS1470, -0.814 ± 0.156 for AUS1471, -1.95 ± 1.60 for AUS1472, -1.87 ± 1.82 for AUS1473, -1.59 ± 1.73 for AUS1474, -3.06 ± 0.85 for AUS1475, -2.79 ± 1.63 for AUS1476/AUS1493, -1.16 ± 0.94 for AUS1478, -0.903 ± 0.164 for AUS1479, -1.64 ± 0.18 for AUS1489, -1.58 ± 1.15 for AUS1490, -2.94 ± 0.78 for AUS1443, and -3.10 ± 0.70 for AUS1322. The body weight change was comparable with the saline group for all treatment groups. The results showed that the antiviral effects of the modified oligonucleotides were much better than the AUS1233/AUS1138.

Example 7: Acute Toxicity Test of modified oligonucleotides in C57BL/6 mice

[0818] This study evaluated the acute toxic effect of modified oligonucleotides on the serum alanine aminotransferase (ALT) levels in C57BL/6 mice. The modified oligonucleotides used in this study included AUS1233/AUS1138, AUS1322, AUS1465, AUS1466/AUS1495, AUS1467, AUS1468, AUS1469, AUS1470, AUS1471, AUS1472, AUS1473, AUS1474, AUS1475, AUS1476/AUS1493, AUS1477, AUS1478, AUS1479, AUS1488/AUS1497, AUS1489, AUS1490. Each compound was dosed three times on Day 0, 2, and 4 at 60 mg/kg by SC. Normal saline was administered as negative control. The ALT levels were measured in the serum samples by enzyme-linked immune-sorbent assays (ELISA).

[0819] Male C57BL/6 mice were purchased from Shanghai SLAC Laboratory Animal CO., Ltd (China). The mice were maintained under specific pathogen-free condition in the BSL-

2+ animal facility of Hagnzhou Eyoung Biotechnology Co., Ltd (China). The methods and purposes of animal experiments are in accordance with the ethical standards and international practices.

[0820] ALT ELISA kits were purchased from Elabscience Biotechnology Co., Ltd (China). Normal saline was purchased from Beijing Sanyao Science & Technology Development (China). ELISA was performed using Enzyme Immunoassay Analyzer of Autobio. Centrifugation of blood was performed with a Preparative Ultracentrifuge of Thermo Fisher Scientific (USA).

[0821] The dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 10 mg/ml, which was diluted with saline again at a target concentration of 6 mg/ml.

[0822] The overall study design is shown in FIG. 8. Male C57BL/6 WT mice were randomly divided into each group. Each group was dosed with one compound of triple doses at 60 mg/kg and a dose volume of 10 ml/kg of body weight or an equal volume of normal saline as control. All mice were dosed at the animal laboratory of Eyoung via SC.

[0823] Approximately 80 μ l of blood sample was taken from a thigh vein on 5, 7, 10, 14, 17 and 21 days after dosing. Blood samples were placed in an incubator at 37°C for approximately 30 minutes, and then centrifuged in a precooled (0-4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further analysis.

[0824] The maximum serum ALT levels was 30.4 ± 14.8 for AUS1233/AUS1138, 335 ± 13 for AUS1322, 10.7 ± 0.5 for S, 10.6 ± 1.4 for AUS1466/AUS1495, 11.8 ± 0.9 for AUS1467, 11.8 ± 3.8 for AUS1468, 9.16 ± 1.69 for AUS1469, 12.5 ± 1.1 for AUS1470, 11.8 ± 2.3 for AUS1471, 12.5 ± 1.1 for AUS1472, 16.6 ± 8.3 for AUS1473, 12.8 ± 2.5 for AUS1474, 13.5 ± 2.4 for AUS1475, 14.7 ± 2.6 for AUS1476/AUS1493, 15.1 ± 1.1 for AUS1477, 14.2 ± 0.6 for AUS1478, 13.1 ± 3.7 for AUS1479, 10.3 ± 1.3 for AUS1488/AUS1497, 14.6 ± 4.5 for AUS1489, 58.4 ± 13.5 for AUS1490. The body weight change was comparable with the saline group for all treatment groups except AUS1322. AUS1322 declined on Day 14 for 3.75 %. The results of the maximum serum ALT levels show that the safety of the modified oligonucleotides are much better than the AUS1233/AUS1138.

Example 8: Antiviral effect of modified oligonucleotides in pcDNA3.1-preS2-GTD HDI-HBV mice

[0825] This study evaluated the effect of modified oligonucleotides on the serum levels of HBsAg in HDI-HBV GTD carrier mice. The modified oligonucleotides used in this study included AUS1233/AUS1138, AUS1463, AUS1476/AUS1493. Each compound was dosed once at 15 or 45 mg/kg by SC. Normal saline was administered as negative control. The HBsAg levels were measured in the serum samples by ELISA.

[0826] Male C57BL/6 mice were purchased from Shanghai SLAC Laboratory Animal CO., Ltd (China). The HDI-HBV mouse model was constructed by hydrodynamic-injection a pcDNA3.1-preS2-GTD plasmid containing preS2 of HBV sequence. HBsAg-positive mice were used Day 23 after the injection. The mice were maintained under specific pathogen-free conditions in the BSL-2+ animal facility of Hagnzhou Eyoung biotechnology Co., Ltd (China). The methods and purposes of animal experiments are in accordance with the ethical standards and international practices.

[0827] HBsAg ELISA kits were purchased from Autobio Diagnostics (China). Normal saline was purchased from Beijing Sanyao Science & Technology Development (China). ELISA was performed using Chemiluminescence immunoassay analyzer (Instrument model: LUMO) of Autobio. Centrifugation of blood was performed with a Preparative Ultracentrifuge of Thermo Fisher Scientific (USA).

[0828] The dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 10 mg/ml, which was diluted with saline again at a target concentration of 1.5 and 4.5 mg/ml.

[0829] The overall study design is shown in FIG. 9. Male HDI-HBV GTD carrier mice were randomly divided into each group. Each group was dosed with one compound at 15 or 45 mg/kg and a dose volume of 10 ml/kg of body weight or an equal volume of normal saline as control. HBsAg-positive mice were dosed 23 days after HDI modeling at the animal laboratory of Eyoung via SC.

[0830] Approximately 80 µl of blood sample was taken from a thigh vein on 0 (before dosing), 3, 7, 10, 14, 21 and 26 days after dosing. Blood samples were placed in an incubator at 37°C for approximately 30 minutes, and then centrifuged in a precooled (0-4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further analysis.

[0831] Serum HBsAg levels in the serum samples were measured using HBsAg ELISA kits (Lot number: 20201116) were purchased from Autobio Diagnostics (China). Briefly, 50 μ l of 25-fold diluted mouse serum or standards was added to a 96-well plate, HBsAg level was measured according to manufacturer's instructions.

[0832] The maximum log₁₀ serum HBsAg reduction was -0.482 ± 0.153 log₁₀ for AUS1233/AUS1138 (15), -0.917 ± 0.258 for AUS1463 (15), -0.891 ± 0.107 for AUS1476/AUS1493 (15), -1.95 ± 1.13 for AUS1233/AUS1138 (45), -2.04 ± 0.28 for AUS1463 (45), and -1.86 ± 0.19 for AUS1476/AUS1493 (45). The body weight change was comparable with the saline group for all treatment groups. The results showed that the antiviral effects of modified oligonucleotides (AUS1463 & AUS1476/AUS1493) were equal or better than the AUS1233/AUS1138 in GTD HDI-HBV mice.

Example 9: Antiviral effect of modified oligonucleotides in pcDNA3.1-preS2-GTA HDI-HBV carrier mice

[0833] This study evaluated the effect of modified oligonucleotides on the serum levels of HBsAg in HDI-HBV carrier mice. The modified oligonucleotides used in this study included AUS1233/AUS1138 & AUS1476/AUS1493. Each compound was dosed once at 15 or 45 mg/kg by SC. Normal saline was administered as negative control. The HBsAg levels were measured in the serum samples by ELISA.

[0834] Male C57BL/6 mice were purchased from Shanghai SLAC Laboratory Animal CO., Ltd (China). The HDI-HBV mouse model was constructed by hydrodynamic-injection a pcDNA3.1-preS2-GTA plasmid containing preS2 of HBV sequence. HBsAg-positive mice were used Day 23 after the injection. The mice were maintained under specific pathogen-free condition in the BSL-2+ animal facility of Hagnzhou Eyoung Biotechnology Co., Ltd (China). The methods and purposes of animal experiments are in accordance with the ethical standards and international practices.

[0835] HBsAg ELISA kits were purchased from Autobio Diagnostics (China). Normal saline was purchased from Beijing Sanyao Science & Technology Development (China). ELISA was performed using Chemiluminescence immunoassay analyzer (Instrument model: LUMO) of Autobio. Centrifugation of blood was performed with a Preparative Ultracentrifuge of Thermo Fisher Scientific (USA).

[0836] The dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 10 mg/ml, which was diluted with saline again at a target concentration of 1.5 and 4.5 mg/ml.

[0837] The overall study design is shown in FIG. 10. Male HDI-HBV carrier mice were randomly divided into each group. Each group was dosed with one compound at 15 or 45 mg/kg and a dose volume of 10 ml/kg of body weight or an equal volume of normal saline as control. HBsAg-positive mice were dosed 23 days after HDI modeling at the animal laboratory of Eyoung via SC.

[0838] Approximately 80 μ l of blood sample was taken from a thigh vein on 0 (before dosing), 3, 7, 10, 14, 21 and 26 days after dosing. Blood samples were placed in an incubator at 37°C for approximately 30 minutes, and then centrifuged in a precooled (0-4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further analysis.

[0839] Serum HBsAg levels in the serum samples were measured using HBsAg ELISA kits (Lot number: 20201116) were purchased from Autobio Diagnostics (China). Briefly, 50 μ l of 20-fold diluted mouse serum or standards was added to a 96-well plate, HBsAg level was measured according to manufacturer's instructions.

[0840] The maximum log₁₀ serum HBsAg reduction was -0.304 ± 0.077 log₁₀ for AUS1233/AUS1138 (15), -0.592 ± 0.094 for AUS1476/AUS1493 (15), -0.842 ± 0.127 for AUS1233, AUS1138 (45), and -1.76 ± 0.10 for AUS1476/AUS1493 (45). The body weight change was comparable with the saline group for all treatment groups. The results showed that the efficacy of different doses of AUS1476/AUS1493 were better than the AUS1233/AUS1138 in GTA HDI-HBV carrier mice.

Example 10: Antiviral effect of modified oligonucleotides in genotype A HBV transgenic mice

[0841] This study evaluated the effect of modified oligonucleotides on the serum levels of HBsAg in HBV transgenic mice. The modified oligonucleotides used in this study included AUS1233/AUS1138 and AUS1476/AUS1493. Each compound was dosed once at 45 mg/kg by SC. Normal saline and AUS1233/AUS1138 were administered as negative control and positive control, respectively. The HBsAg levels and HBeAg levels were measured in the serum samples by enzyme-linked immune-sorbent assays (ELISA). The maximum log₁₀ serum HBsAg reduction was -1.768 ± 0.16 log₁₀ for AUS1233/AUS1138, and -2.483 ± 0.27 for

AUS1476/AUS1493. The body weight change was comparable with the saline group for all treatment groups.

[0842] HBV transgenic mice, prepared from male and female C57BL/6 mice of 5-7 weeks old with 1.2-fold length of HBV A subtype genome, stably express HBsAg in liver. The mice were obtained from Xiamen University and bred under specific pathogen-free condition in the BSL-2+ animal facility of Zhejiang Chinese Medical University (China). The methods and purposes of animal experiments are in accordance with the ethical standards and international practices.

[0843] HBsAg ELISA kits were purchased from Autobio Diagnostics (China). HBeAg ELISA kits were purchased from Beijing BGI-BGIN Biotech (China). Normal saline was purchased from Beijing Sanyao Science & Technology Development (China). ELISA was performed using Chemiluminescence immunoassay analyzer (Instrument model: LUMO) of Autobio and Microplate reader of Pulangbio. Centrifugation of blood was performed with a Preparative Ultracentrifuge of Thermo Fisher Scientific (USA).

[0844] The dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 10 mg/ml, which was diluted with saline again at a target concentration of 4.5 mg/ml.

[0845] The overall study design is shown in FIG. 11. Male and female HBV transgenic mice were randomly divided into each group. Each group was dosed with one compound at 45 mg/kg and a dose volume of 10 ml/kg of body weight or an equal volume of normal saline as control. All mice were dosed at the animal laboratory of Eyoung via SC.

[0846] Approximately 80 μ l of blood sample was taken from a thigh vein on 0 (before dosing), 3, 7, 10, 14, 21 and 29 days after dosing. Blood samples were placed in an incubator at 37°C for approximately 30 minutes, and then centrifuged in a precooled (0-4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further analysis.

[0847] Serum HBsAg levels in the serum samples were measured using HBsAg ELISA kits (Lot number: 20201116) were purchased from Autobio Diagnostics (China). Briefly, 50 μ l of 20-fold diluted mouse serum or standards was added to a 96-well plate, HBsAg level was measured according to manufacturer's instructions.

[0848] The maximum log₁₀ serum HBsAg reduction was -1.79 ± 0.16 log₁₀ for **AUS1233/AUS1138**, -2.25 ± 0.28 for **AUS1463**, and -2.21 ± 0.27 for **AUS1476/AUS1493**. The

results showed that the efficacy of **AUS1476/AUS1493** is better than the **AUS1233/AUS1138** in GTA HBV transgenic mice.

Example 11: Antiviral effect of modified oligonucleotides in AAV-HBV carrier mice (GTD)

[0849] This study evaluated the effect of modified oligonucleotides on the serum levels of HBsAg in AAV HBV mice. HBV HDI mice model based on the injection of AAV-HBV plasmid into male C57BL/6 mice. The modified oligonucleotides used in this study included **AUS1233/AUS1138** and **AUS1476/AUS1493**. Each compound was dosed once at 15 mg/kg, 45 mg/kg and 90mg/kg respectively by SC. Normal saline and **AUS1233/AUS1138** were administered as negative control and positive control, respectively. The HBsAg, HBeAg and ALT levels was measured in the serum samples by ELISA. The HBV DNA levels was measured in the serum samples by real-time PCR using a Taqman probe.

[0850] Male AAV HBV-GTD C57BL/6 mice were from The First Hospital of Zhejiang University. The HDI-HBV mouse model was constructed by injection a 1×10^{11} vg AAV-GTD plasmid containing HBV sequence. The mice were maintained under specific pathogen-free condition in the BSL-2+ animal facility of The First Hospital of Zhejiang University. The methods and purposes of animal experiments are in accordance with the ethical standards and international practices.

[0851] HBsAg ELISA kits were purchased from Autobio Diagnostics (China). ALT and AST ELISA kits were purchased from Elabscience Biotechnology Co., Ltd (China). Normal saline was purchased from Beijing Sanyao Science & Technology Development (China). ELISA was performed using Chemiluminescence immunoassay analyzer (Instrument model: LUMO) of Autobio. Centrifugation of blood was performed with a Preparative Ultracentrifuge of Thermo Fisher Scientific (USA).

[0852] The dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 10 mg/ml, which was diluted with saline again at a target concentration of 1.5 mg/ml, 4.5 mg/ml and 9 mg/ml.

[0853] Animal Dosing. The overall study design is shown in FIG. 12. Male AAV-HBV carrier mice were randomly divided into each group. Mice were dosed with one compound at 15 mg/kg, 45 mg/kg and 90 mg/kg respectively. A dose volume of 10 ml/kg of body weight or an equal volume of normal saline as control.

[0854] Approximately 80 μ l of blood sample was taken from a thigh vein on 0 (before dosing), 3, 7, 10, 14, 21 and 28 days after dosing. Blood samples were placed in an incubator at 37 °C for approximately 30 minutes, and then centrifuged in a precooled (0-4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further analysis.

[0855] Serum HBsAg levels in the serum samples were measured using HBsAg ELISA kits (Lot number: 20201116) were purchased from Autobio Diagnostics (China). Briefly, 50 μ l of 20-fold diluted mouse serum or standards was added to a 96-well plate, HBsAg level was measured according to manufacturer's instructions.

[0856] The maximum log₁₀ serum HBsAg reduction was -0.413 ± 0.309 log₁₀ for AUS1233/AUS1138 (15), -0.736 ± 0.481 for AUS1476/AUS1493 (15), -1.274 ± 0.456 for AUS1233/AUS1138 (45), -1.713 ± 0.585 for AUS1476/AUS1493, -1.724 ± 0.194 for AUS1233/AUS1138 (90), -2.394 ± 0.408 for AUS1476/AUS1493. The results showed that the efficacy of AUS1476/AUS1493 is better than the AUS1233/AUS1138 in AAV-HBV carrier mice (GTD).

Example 12: Acute toxic effect of modified oligonucleotides in C57BL/6 mice

[0857] This study evaluated the acute toxic effect of modified oligonucleotides on the serum ALT and AST levels in C57BL/6 mice. The modified oligonucleotides used in this study included AUS1233/AUS1138, AUS1463, AUS1476/AUS1493. Each compound was dosed three times on Day 0, 2, and 4 at 60 mg/kg by SC. Normal saline was used as a negative control. The blood routine was measured in the whole blood samples by HITACHI AUTOMATIC ANALYZER 3100. The ALT and AST levels were measured in the serum samples by ELISA.

[0858] Male C57BL/6 mice were purchased from Shanghai SLAC Laboratory Animal CO., Ltd (China). The mice were maintained under specific pathogen-free condition in the BSL-2+ animal facility of Hagnzhou Eyoung Biotechnology Co., Ltd (China). The methods and purposes of animal experiments are in accordance with the ethical standards and international practices.

[0859] ALT and ELISA kits were purchased from Elabscience Biotechnology Co., Ltd (China). Normal saline was purchased from Beijing Sanyao Science & Technology Development (China). ELISA was performed using Enzyme Immunoassay Analyzer of Autobio. Centrifugation of blood was performed with a Preparative Ultracentrifuge of Thermo Fisher Scientific (USA).

[0860] The dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 20 mg/ml, which was diluted with saline again at a target concentration of 6 mg/ml and 15 mg/ml.

[0861] Animal Dosing--The overall study design is shown in FIG. 13. Male C57BL/6 mice were randomly divided into each group. Each compound was dosed three times on Day 0, 2, and 4 at 60 mg/kg by subcutaneous injection (SC) and a dose volume of 10 µl/g of body weight or an equal volume of normal saline as control.

[0862] Approximately 80 µl of blood sample was taken from a thigh vein on 7, 14, 17, 21 and 27 days after dosing. Blood samples were placed in an incubator at 37°C for approximately 30 minutes, and then centrifuged in a precooled (0-4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further analysis.

[0863] Approximately 50 µl of blood sample was taken from a thigh vein on Day 17 after dosing. Blood samples were anticoagulated by EDTA-2Na anticoagulant and diluted. The obtained anticoagulant blood dilution samples were stored in a 4°C refrigerator for blood routine analysis.

[0864] Assays--Serum ALT levels were measured using ALT ELISA kits (Number: E-BC-K235-M) were purchased from Elabscience Biotechnology Co., Ltd (China). Briefly, 50µl of 2-fold diluted mouse serum or standards was added to a 96-well plate, HBsAg level was measured according to manufacturer's instructions.

[0865] The results of serum ALT and AST levels show the present modified oligonucleotides have a higher safety level than the AUS1233/AUS1138.

Example 13: Antiviral effect of eASO compounds in HepG2.2.15 cells

[0866] The antiviral effect of eASO compounds was evaluated by measuring the levels of hepatitis B virus (HBV) surface antigen (HBsAg) and HBV e antigen (HBeAg) in HepG2.2.15 cells. The HBsAg levels were measured by chemiluminescence immunoassays, the HBeAg levels were measured by ELISA assays and cell viability was determined by MTT cell proliferation assay. The calculation result was expressed as mean and SD.

[0867] HepG2.2.15 cells that can stably express HBsAg and HBeAg were purchased from China Center for Type Culture Collection (CCTCC No. GDC0141). The eASO compound solutions were prepared by dissolving each compound in saline or RNase-free double distilled H₂O (dd H₂O) to make a target stock solution at 50 µM.

[0868] The eASO compounds were set in a series of concentrations (0, 0.037, 0.11, 0.34, 1, 3.1, 9.3, 27.8, 83.3, 250 nM) and worked with HepG2.2.15 cells by reverse transfection. First, the 96-well cell culture plate was covered with 50 ul Rat Tail Collagen I in every well and removed one hour later. Second, P3000 and Lipofectamine® 3000 Reagent was diluted with Opti-MEM™ reduced serum medium and mixed with the serial dilution compound solution, followed by incubation at room temperature for 10 minutes. Third, 10 ul of transfection reagent mixture of P3000, Lipo3000 and eASO and 100ul of cell suspension with a concentration of 3.5×10^5 cells were added to each well.

[0869] The mixture was pipetted 4 or 5 times and the plate was placed in a 37°C incubator. After reverse transfection 24h, the media was exchanged with fresh cell culture medium. Following a 48h culture, supernatant was collected for detection. The remaining cells were used for cell viability test. The resulting data is represented in **Table 14.1**, as EC50(nM) and MTT CC25 (nM). The EC50(nM) ratio was calculated by dividing the EC50 value determined following the treatment with the modified oligonucleotide of any one of AUS1010 to AUS1714 (SEQ ID NOs: 11 to 666) by the EC50 value determined following the treatment with a reference oligonucleotide of AUS1233 (SEQ ID NO: 10). A lower EC50 ratio value suggests the increased ability of the modified oligonucleotide to decrease HBsAg levels relative to the reference oligonucleotide. The MTT CC25 (nM) ratio was calculated by dividing the MTT CC25 value determined following treatment with the modified oligonucleotide of any one of AUS1010 to AUS1714 (SEQ ID NOs: 11 to 666) by the MTT CC25 value determined following the treatment with a reference oligonucleotide of AUS1233 (SEQ ID NO: 10). A higher MTT CC25 ratio suggests an increased cellular viability relative to the reference oligonucleotide. The C/E ratio was calculated by dividing the MTT CC25 ratio value by the EC50 value. A higher C/E ratio suggests a balanced decrease in HBsAg levels while maintaining high cellular viability.

Example 14: Antiviral effect of eASO compounds in HepG2.2.15 cells

[0870] The antiviral effect of eASO compounds was evaluated by measuring the levels of hepatitis B virus (HBV) surface antigen (HBsAg) and HBV e antigen (HBeAg) in HepG2.2.15 cells. The HBsAg levels were measured by Chemiluminescence immunoassay assays, the HBeAg levels were measured by ELISA assays and cell viability was determined by CCK-8 cell proliferation assay. The calculation result was expressed as mean and SD.

[0871] HepG2.2.15 cells that can stably express HBsAg and HBeAg were purchased from China Center for Type Culture Collection (CCTCC No. GDC0141). The eASO compound solutions were prepared by dissolving each compound in saline or RNase-free double distilled H₂O (dd H₂O) to make a target stock solution at 50 μ M.

[0872] The eASO compounds were set in a series of concentrations (0, 0.037, 0.11, 0.34, 1, 3.1, 9.3, 27.8, 83.3, 250 nM) and worked with HepG2.2.15 cells by reverse transfection. First, the 96-well cell culture plate was covered with 50ul Rat Tail Collagen I in every well and removed one hour later. Second, P3000 and Lipofectamine® 3000 Reagent were diluted with Opti-MEM™ reduced serum medium and mixed with the serial dilution compound solution, and allowed to incubate at room temperature for 10 minutes. Third, 10 ul of transfection reagent mixture of P3000, Lipo3000 and eASO and 100ul of cell suspension with a concentration of 3.5×10^5 cells were added to each well. The mixture was pipetted 4 or 5 times and the plate was placed in a 37°C incubator. After reverse transfection 24h, media was exchanged with fresh cell culture medium. After 48h supernatant was collected for HBsAg detection. For cell viability test, cell supernatant was removed after reverse transfection 48h, and the CCK-8 reagent was added to detect the absorbance. The resulting data is represented in Table 14.1, EC₅₀(nM) ratio was calculated by dividing the EC₅₀ value determined following the treatment with the modified oligonucleotide of any one of AUS1010 to AUS1714 (SEQ ID NOs: 11 to 666) by the EC₅₀ value determined following the treatment with a reference oligonucleotide of AUS1233 (SEQ ID NO: 10). A lower EC₅₀ ratio value suggests the increased ability of the modified oligonucleotide to decrease HBsAg levels relative to the reference oligonucleotide. The ratio of CCK8 CC₃₀ was calculated by dividing the CCK8 CC₃₀ value determined following treatment with the modified oligonucleotide of any one of AUS1010 to AUS1714 (SEQ ID NOs: 11 to 666) by the CCK8 CC₃₀ value determined following the treatment with a reference oligonucleotide of AUS1233 (SEQ ID NO: 10). A higher CCK8 CC₃₀ ratio suggests an increased cellular viability relative to the reference oligonucleotide. The C/E ratio was calculated by dividing the CCK8 CC₃₀ ratio value by the EC₅₀ value. A higher C/E ratio suggests a balanced decrease in HBsAg levels while maintaining high cellular viability.

Table 14.1: Antiviral effect of eASO compounds in HepG2.2.15 cells by MMT and/or CCK8 proliferation assays

SEQ ID NO:	ID	Ratio EC ₅₀	Ratio MTT CC25 or CCK8 CC30	C/E ratio
12	AUS1011	2.9	0.1	0.050
13	AUS1012	>250	>250	
14	AUS1013	39.19	>250	
15	AUS1014	>250	>250	
16	AUS1015	1.84	>250	
18	AUS1017	5.5	2.6	0.47
33	AUS1036	10.75	2.30	0.21
34	AUS1037	1.69	2.23	1.32
35	AUS1038	1.53	1.39	0.91
36	AUS1039	8.14	2.35	0.29
37	AUS1042	2.38	1.57	0.66
38	AUS1043	1.41	1.51	1.07
39	AUS1044	2.47	1.09	0.44
40	AUS1045	3.09	1.49	0.48
41	AUS1046	3.65	2.13	0.58
42	AUS1049	1.42	2.11	1.49
43	AUS1050	0.90	1.65	1.83
44	AUS1051	1.66	2.14	1.30
45	AUS1052	1.54	1.18	0.77
46	AUS1053	1.86	1.16	0.62
47	AUS1054	2.16	0.89	0.41
48	AUS1055	2.25	0.54	0.24
49	AUS1056	2.32	1.51	0.65
50	AUS1057	1.33	0.80	0.60
51	AUS1058	1.64	0.95	0.58
52	AUS1059	2.14	0.75	0.35
53	AUS1060	2.19	1.33	0.61
54	AUS1061	1.05	0.82	0.78
55	AUS1062	2.84	2.09	0.74
56	AUS1063	5.26	1.88	0.36
57	AUS1064	6.19	2.45	0.40
58	AUS1065	2.50	1.66	0.66
59	AUS1066	2.50	2.28	0.91
60	AUS1067	3.67	1.60	0.44
61	AUS1068	2.61	1.50	0.57
62	AUS1069	1.72	1.11	0.64
63	AUS1070	1.90	1.43	0.75
64	AUS1071	2.54	2.34	0.92

SEQ ID NO:	ID	Ratio EC ₅₀	Ratio MTT CC25 or CCK8 CC30	C/E ratio
65	AUS1072	2.80	2.36	0.84
66	AUS1073	1.65	1.20	0.72
67	AUS1074	3.41	1.03	0.30
68	AUS1075	5.06		
69	AUS1076	1.55		
70	AUS1077	1.83		
71	AUS1078	2.66	1.26	0.48
72	AUS1079	3.94	1.45	0.37
73	AUS1081	2.95	1.20	0.41
74	AUS1082	2.03	0.54	0.27
75	AUS1083	1.98	0.69	0.35
76	AUS1084	3.71	>250	
77	AUS1085	2.41	>250	
78	AUS1086	3.68	>250	
79	AUS1087	5.59	>250	
80	AUS1088	4.93	>250	
81	AUS1089	2.81	>250	
82	AUS1090	2.07	>250	
83	AUS1091	2.08	0.94	0.45
84	AUS1092	9.62	>250	
85	AUS1093	7.07	>250	
86	AUS1094	2.83	>250	
87	AUS1095	2.42	>250	
88	AUS1096	1.87	>250	
89	AUS1097	1.59	>250	
90	AUS1098	1.70	>250	
91	AUS1099	1.55	>250	
92	AUS1100	1.75	>250	
93	AUS1101	5.73	>250	
94	AUS1102	17.14	>250	
96	AUS1105	1.24	0.88	0.71
97	AUS1106	1.80	1.06	0.59
98	AUS1107	2.63	1.37	0.52
99	AUS1108	1.78	0.93	0.52
100	AUS1109	1.21	0.85	0.71
101	AUS1110	2.16	1.14	0.53
102	AUS1111	2.66	1.64	0.62
103	AUS1112	2.18	1.13	0.52
104	AUS1113	1.82	1.13	0.62
105	AUS1114	1.66	1.09	0.66
106	AUS1116	2.40	1.24	0.52

SEQ ID NO:	ID	Ratio EC ₅₀	Ratio MTT CC25 or CCK8 CC30	C/E ratio
107	AUS1117	1.94	1.11	0.57
108	AUS1118	1.93	1.31	0.68
109	AUS1119	1.92	0.97	0.50
110	AUS1120	2.89	0.72	0.25
111	AUS1121	3.15	3.54	1.12
112	AUS1122	3.12	0.54	0.17
113	AUS1123	2.31	0.98	0.42
114	AUS1124	1.02	0.59	0.58
115	AUS1125	2.08	1.45	0.69
116	AUS1126	1.19	0.82	0.69
117	AUS1127	1.43	0.76	0.53
118	AUS1128	1.46	0.90	0.62
119	AUS1129	1.62	1.02	0.63
120	AUS1130	1.68	0.85	0.50
121	AUS1131	2.06	0.92	0.45
122	AUS1132	4.88	2.41	0.49
123	AUS1133	3.15	1.16	0.37
124	AUS1134	1.55	0.89	0.57
125	AUS1135	1.68	0.90	0.54
126	AUS1136	1.54	0.87	0.57
127	AUS1137	1.63	0.80	0.49
132	AUS1143	1.28	0.81	0.63
133	AUS1144	2.16	0.66	0.31
134	AUS1145	1.95	0.64	0.33
135	AUS1146	2.15	0.60	0.28
136	AUS1147	2.14	0.59	0.27
137	AUS1148	1.93	0.98	0.51
138	AUS1149	1.80	0.83	0.46
139	AUS1151	1.11	0.82	0.74
140	AUS1152	2.43	1.43	0.59
141	AUS1153	3.35	1.58	0.47
142	AUS1154	2.67	3.05	1.14
143	AUS1156	9.465422	2.729780304	0.288395
144	AUS1157	9.9710755	2.654861737	0.266256
145	AUS1158	23.050224	3.163074364	0.137225
146	AUS1159	19.57928	2.076928408	0.106078
147	AUS1160	15.571917	2.499341604	0.160503
148	AUS1161	32.027347	2.46586735	0.076993
149	AUS1162	9.5	2.7	0.29
150	AUS1163	10.0	2.7	0.27
151	AUS1164	23.1	3.2	0.14

SEQ ID NO:	ID	Ratio EC ₅₀	Ratio MTT CC25 or CCK8 CC30	C/E ratio
152	AUS1165	19.6	2.1	0.11
154	AUS1168	3.63	1.11	0.31
155	AUS1169	2.90	1.17	0.40
156	AUS1170	3.98	1.26	0.32
157	AUS1171	3.51	1.25	0.35
159	AUS1174	0.65	0.93	1.42
160	AUS1175	1.27	1.17	0.92
161	AUS1176	3.05	1.60	0.52
163	AUS1178	1.14	1.06	0.94
164	AUS1179	1.03	0.64	0.62
165	AUS1180	1.42	0.50	0.35
166	AUS1181	1.48	0.69	0.46
167	AUS1182	1.35	0.79	0.59
168	AUS1183	2.97	0.60	0.20
169	AUS1184	2.52	0.64	0.25
170	AUS1185	2.52	0.81	0.32
171	AUS1186	2.88	0.88	0.30
172	AUS1187	1.17	0.65	0.55
173	AUS1188	1.11	0.76	0.68
174	AUS1189	1.51	0.41	0.27
175	AUS1190	0.94	0.64	0.68
176	AUS1191	1.34	0.91	0.68
177	AUS1192	1.24	0.57	0.46
178	AUS1193	3.30	0.84	0.26
179	AUS1194	4.54	1.01	0.22
181	AUS1198	2.2	>250	
182	AUS1199	6.1	0.3	0.05
183	AUS1200	2.7	>250	
184	AUS1201	6.1	0.2	0.04
185	AUS1202	0.7	0.3	0.44
186	AUS1203	1.8	>250	
187	AUS1204	1.0	0.3	0.32
188	AUS1205	4.3	>250	
189	AUS1206	2.16	1.71	0.79
190	AUS1207	1.20	1.68	1.41
191	AUS1208	1.29	2.02	1.56
192	AUS1209	6.78	>250	
193	AUS1210	22.96	>250	
194	AUS1211	>250	>250	
195	AUS1212	2.2	0.9	0.39
196	AUS1213	1.6	0.7	0.41

SEQ ID NO:	ID	Ratio EC ₅₀	Ratio MTT CC25 or CCK8 CC30	C/E ratio
197	AUS1214	1.3	0.6	0.50
198	AUS1215	1.1	0.4	0.35
199	AUS1216	1.6	0.4	0.25
200	AUS1217	1.8	0.3	0.16
201	AUS1218	1.2	0.5	0.44
202	AUS1219	0.9	0.3	0.30
203	AUS1220	1.7	0.6	0.35
205	AUS1224	2.7	1.2	0.46
206	AUS1225	8.8	2.2	0.26
207	AUS1226	19.1	3.0	0.16
208	AUS1227	21.4	2.8	0.13
209	AUS1228	32.4	4.4	0.14
211	AUS1232			
213	AUS1234	7.0	0.7	0.10
214	AUS1235	3.4	0.6	0.17
215	AUS1236	1.6	0.7	0.44
216	AUS1237	2.4	0.5	0.21
217	AUS1238	4.0	0.4	0.10
218	AUS1241	0.10	2.08	20.64
219	AUS1242	1.16	1.16	1.00
220	AUS1243	0.79	1.14	1.44
221	AUS1244	0.37	1.04	2.82
222	AUS1245	1.20	0.97	0.82
223	AUS1246	0.84	0.92	1.10
224	AUS1247	1.18	0.39	0.33
225	AUS1248	1.47	0.49	0.33
226	AUS1249	0.96	0.75	0.78
227	AUS1250	1.30	0.44	0.34
228	AUS1251	1.23	0.53	0.43
229	AUS1252	0.72	0.71	0.99
230	AUS1253	0.45	0.59	1.33
231	AUS1254	0.39	0.42	1.09
232	AUS1255	0.50	0.41	0.83
233	AUS1256	0.52	0.64	1.25
234	AUS1257	0.97	0.93	0.96
235	AUS1258	1.26	0.87	0.69
236	AUS1259	0.76	0.73	0.95
237	AUS1260	0.61	0.82	1.34
238	AUS1261	1.10	1.11	1.02
239	AUS1262	0.94	1.16	1.24
240	AUS1263	0.50	1.04	2.09

SEQ ID NO:	ID	Ratio EC ₅₀	Ratio MTT CC25 or CCK8 CC30	C/E ratio
241	AUS1264	0.45	0.96	2.12
242	AUS1266	2.04	3.37	1.65
243	AUS1267	1.25	0.98	0.78
244	AUS1268	1.81	1.18	0.65
245	AUS1269	1.06	1.06	1.00
246	AUS1270	1.52	1.31	0.86
247	AUS1271	2.03	1.38	0.68
248	AUS1272	2.78	1.34	0.48
249	AUS1274	1.32	0.45	0.34
250	AUS1275	0.97	0.34	0.35
251	AUS1277	0.48	0.81	1.69
252	AUS1278	0.29	1.02	3.48
253	AUS1279	0.55	0.72	1.31
254	AUS1280	0.51	0.67	1.31
255	AUS1281	0.40	0.95	2.39
256	AUS1282	0.29	0.85	2.92
257	AUS1283	0.21	0.58	2.73
258	AUS1284	0.28	0.92	3.22
259	AUS1285	0.18	0.61	3.35
260	AUS1286	0.38	0.66	1.75
261	AUS1287	0.38	0.94	2.44
262	AUS1288	0.21	0.98	4.65
263	AUS1289	0.30	0.62	2.06
264	AUS1290	0.25	0.84	3.41
265	AUS1291	0.11	0.56	5.05
266	AUS1292	0.15	0.58	3.88
267	AUS1293	0.18	0.65	3.63
268	AUS1294	0.12	0.47	3.74
269	AUS1295	0.14	0.44	3.04
270	AUS1296	0.17	0.58	3.44
271	AUS1298	1.37	1.03	0.75
272	AUS1299	1.88	1.18	0.62
273	AUS1300	1.04	0.89	0.85
275	AUS1302	1.55	0.88	0.57
276	AUS1303	0.96	1.19	1.24
277	AUS1304	1.02	1.49	1.46
278	AUS1305	1.33	1.78	1.34
279	AUS1306	1.46	1.12	0.77
280	AUS1307	1.78	1.01	0.57
281	AUS1308	1.23	1.02	0.83
282	AUS1309	1.89	1.11	0.59

SEQ ID NO:	ID	Ratio EC ₅₀	Ratio MTT CC25 or CCK8 CC30	C/E ratio
283	AUS1310	1.79	0.98	0.55
284	AUS1311	1.86	1.00	0.54
285	AUS1312	1.34	1.26	0.94
286	AUS1313	1.67	1.45	0.87
287	AUS1314	2.13	1.18	0.56
288	AUS1315	1.46	1.21	0.83
289	AUS1316	1.91	1.42	0.74
290	AUS1317	1.63	1.22	0.75
291	AUS1318	1.83	1.19	0.65
292	AUS1319	1.38	1.17	0.84
293	AUS1320	2.08	1.43	0.69
294	AUS1322	0.76	1.15	1.52
295	AUS1323	0.94	1.32	1.41
296	AUS1324	1.12	1.30	1.16
298	AUS1326	0.14	0.92	6.60
299	AUS1327	0.11	0.98	9.20
300	AUS1328	0.10	1.00	9.79
301	AUS1329	0.09	0.88	9.97
302	AUS1332	0.12	3.04	25.45
303	AUS1333	0.6	0.6	0.90
304	AUS1334	0.7	0.6	0.90
305	AUS1335	0.8	1.3	1.53
306	AUS1336	0.8	1.7	2.05
307	AUS1337	0.4	1.3	3.04
308	AUS1338	0.3	0.8	2.59
309	AUS1340	0.29	0.80	2.80
310	AUS1341	0.26	0.70	2.64
311	AUS1342	0.40	0.63	1.58
312	AUS1343	0.37	0.85	2.31
313	AUS1344	0.29	0.83	2.84
314	AUS1345	0.24	0.65	2.63
315	AUS1346	0.15	0.59	3.94
316	AUS1347	0.22	0.44	2.01
317	AUS1348	0.22	0.84	3.80
318	AUS1349	0.14	0.59	4.34
319	AUS1350	0.15	0.57	3.80
320	AUS1351	0.23	0.66	2.89
321	AUS1352	0.16	0.73	4.59
322	AUS1353	0.15	0.51	3.48
323	AUS1354	0.12	0.40	3.44
324	AUS1355	0.13	0.60	4.61

SEQ ID NO:	ID	Ratio EC ₅₀	Ratio MTT CC25 or CCK8 CC30	C/E ratio
325	AUS1356	0.19	0.73	3.80
326	AUS1357	0.14	0.56	4.02
327	AUS1358	0.12	0.60	4.86
328	AUS1359	0.32	0.71	2.23
329	AUS1360	0.09	0.80	8.61
330	AUS1361	0.13	0.89	6.85
331	AUS1362	0.10	0.99	9.71
332	AUS1365	0.08	1.94	24.34
333	AUS1366	24.42	>250	
334	AUS1367	0.23	0.63	2.72
336	AUS1370	0.74	1.10	1.49
337	AUS1371	1.66	1.47	0.89
338	AUS1372	0.92	1.44	1.56
339	AUS1373	0.24	1.34	5.62
340	AUS1374	0.74	1.12	1.51
341	AUS1375	1.02	0.89	0.87
342	AUS1376	0.56	0.93	1.66
343	AUS1377	0.27	1.06	3.93
344	AUS1378	0.48	0.93	1.93
345	AUS1379	0.77	0.69	0.89
346	AUS1382	0.18	1.06	5.99
347	AUS1383	0.13	0.97	7.39
348	AUS1384	0.15	1.03	6.86
349	AUS1385	0.23	0.90	3.98
350	AUS1387	1.29	0.82	0.64
351	AUS1388	0.09	0.92	9.72
352	AUS1389	0.13	0.71	5.31
353	AUS1390	0.15	0.76	4.92
354	AUS1391	0.19	0.76	3.96
355	AUS1392	0.25	0.71	2.81
356	AUS1393	0.27	0.70	2.59
357	AUS1394	0.24	0.87	3.58
358	AUS1395	0.17	0.97	5.76
359	AUS1396	0.13	0.95	7.48
372	AUS1409	0.04	1.61	38.05
373	AUS1410	0.07	1.25	17.88
374	AUS1411	0.08	0.83	10.18
375	AUS1412	0.06	0.88	14.00
376	AUS1413	0.07	0.84	12.46
377	AUS1414	0.07	0.72	10.98
378	AUS1415	0.06	0.75	13.29

SEQ ID NO:	ID	Ratio EC ₅₀	Ratio MTT CC25 or CCK8 CC30	C/E ratio
385	AUS1422	0.10	0.99	10.07
386	AUS1423	0.07	0.99	13.47
387	AUS1424	0.08	0.97	12.62
388	AUS1425	0.10	0.95	9.21
389	AUS1426	0.08	0.83	10.02
390	AUS1427	0.07	0.77	11.07
391	AUS1428	0.08	0.92	11.95
392	AUS1429	0.08	1.22	15.74
393	AUS1430	0.07	1.19	17.15
394	AUS1431	0.05	1.04	19.01
395	AUS1432	0.05	1.19	23.71
396	AUS1433	0.10	1.09	10.92
397	AUS1434	0.76	1.15	1.52
398	AUS1435	0.18	1.22	6.70
399	AUS1436	0.24	1.11	4.65
400	AUS1437	0.37	1.33	3.63
401	AUS1438	0.15	0.89	5.76
402	AUS1439	0.34	1.25	3.72
403	AUS1440	0.18	1.23	6.75
404	AUS1441	0.70	1.31	1.85
406	AUS1443	0.18	0.96	5.25
407	AUS1444	0.22	0.93	4.17
408	AUS1445	0.25	1.01	4.03
409	AUS1446	0.40	1.46	3.67
410	AUS1447	0.52	1.11	2.12
411	AUS1448	0.67	1.07	1.59
412	AUS1449	0.74	1.04	1.41
413	AUS1450	0.96	1.26	1.32
414	AUS1451	0.70	1.22	1.75
415	AUS1452	0.54	1.09	2.00
416	AUS1453	0.84	1.22	1.46
417	AUS1454	0.65	1.00	1.52
418	AUS1455	0.59	1.06	1.80
419	AUS1456	0.56	1.08	1.91
420	AUS1457	0.53	1.15	2.15
421	AUS1458	0.22	0.95	4.38
422	AUS1459	0.25	1.08	4.33
423	AUS1460	0.23	1.29	5.71
424	AUS1461	0.40	1.75	4.41
425	AUS1462	0.25	1.77	7.14
426	AUS1463	0.27	1.74	6.37

SEQ ID NO:	ID	Ratio EC ₅₀	Ratio MTT CC25 or CCK8 CC30	C/E ratio
427	AUS1464	0.38	2.10	5.45
428	AUS1465	0.43	1.32	3.03
429	AUS1466	0.19	1.20	6.15
430	AUS1467	0.24	1.14	4.72
431	AUS1468	0.25	1.30	5.29
432	AUS1469	0.25	1.22	4.90
433	AUS1470	0.16	1.16	7.09
434	AUS1471	0.18	1.23	7.03
435	AUS1472	0.19	1.31	7.01
436	AUS1473	0.14	1.37	9.49
437	AUS1474	0.16	1.42	9.05
438	AUS1475	0.18	1.31	7.39
439	AUS1476	0.10	1.49	15.50
440	AUS1477	0.20	1.28	6.24
441	AUS1478	0.37	1.33	3.62
442	AUS1479	0.13	1.56	12.00
443	AUS1480	0.14	1.27	8.90
444	AUS1481	0.14	1.05	7.43
445	AUS1482	0.14	0.90	6.63
446	AUS1483	0.14	1.07	7.62
447	AUS1484	0.47	1.24	2.65
448	AUS1485	0.54	1.24	2.29
449	AUS1486	0.76	1.46	1.92
450	AUS1487	0.68	1.63	2.39
451	AUS1488	0.20	1.73	8.62
452	AUS1489	0.18	1.81	10.33
453	AUS1490	0.16	1.77	11.26
455	AUS1492	0.25	1.33	5.32
456	AUS1493	0.38	1.63	4.25
457	AUS1494	0.22	1.47	6.59
458	AUS1495	0.42	1.45	3.42
466	AUS1505	29.7	0.6	0.02
491	AUS1530	>250	>250	
492	AUS1531	>250	>250	
493	AUS1532	>250	>250	
494	AUS1533	>250	>250	
495	AUS1534	>250	>250	
496	AUS1535	35.9	>250	
497	AUS1536	1.71	>250	
498	AUS1537	1.89	>250	
499	AUS1538	3.33	>250	

SEQ ID NO:	ID	Ratio EC ₅₀	Ratio MTT CC25 or CCK8 CC30	C/E ratio
500	AUS1539	5.75	>250	
501	AUS1540	3.29	>250	
502	AUS1541	1.69	>250	
503	AUS1542	1.04	>250	
504	AUS1543	3.98	>250	
505	AUS1544	>250	>250	
506	AUS1545	35.96	>250	
507	AUS1546	1.65	0.76	0.46
508	AUS1547	>250	>250	
509	AUS1548	>250	>250	
510	AUS1549	>250	>250	
511	AUS1550	>250	>250	
512	AUS1551	9.57	>250	
513	AUS1552	6.70	>250	
539	AUS1578	2.9	1.8	0.62
540	AUS1579	2.0	0.5	0.24
541	AUS1580	6.9	1.1	0.16
542	AUS1581	3.2	0.4	0.11
543	AUS1582	2.00	2.82	1.41
544	AUS1583	2.16	0.82	0.38
545	AUS1584	1.08	0.80	0.74
546	AUS1585	1.19	0.82	0.69
547	AUS1586	0.49	0.91	1.88
548	AUS1587	0.94	1.78	1.90
549	AUS1588	4.31	1.07	0.25
550	AUS1589	0.99	1.41	1.42
551	AUS1590	2.86	3.82	1.33
552	AUS1591	>250	5.82	
553	AUS1592	5.7	3.1	0.53
554	AUS1593	4.0	2.6	0.67
555	AUS1594	2.6	1.8	0.68
556	AUS1595	2.7	1.2	0.45
557	AUS1596	2.8	3.5	1.25
558	AUS1597	1.3	1.6	1.21
559	AUS1598	1.9	1.5	0.77
560	AUS1599	1.6	0.4	0.27
561	AUS1600	1.8	0.8	0.46
562	AUS1601	7.5	8.9	1.18
563	AUS1602	1.2	0.6	0.55
564	AUS1603	0.9	0.9	0.99
565	AUS1604	1.3	1.1	0.85

SEQ ID NO:	ID	Ratio EC ₅₀	Ratio MTT CC25 or CCK8 CC30	C/E ratio
566	AUS1605	1.7	7.4	4.40
567	AUS1606	1.1	1.2	1.04
568	AUS1607	1.8	1.8	1.00
569	AUS1608	2.1	3.0	1.44
570	AUS1609	1.0	0.8	0.78
571	AUS1610	1.5	1.1	0.75
572	AUS1611	1.4	1.2	0.86
573	AUS1612	1.0	0.5	0.51
574	AUS1613	1.2	0.7	0.56
575	AUS1614	2.9	1.3	0.46
576	AUS1615	2.4	2.3	0.96
577	AUS1616	1.4	2.6	1.88
578	AUS1617	1.3	1.8	1.32
579	AUS1618	2.3	0.8	0.37
580	AUS1619	5.9	4.3	0.72
581	AUS1620	6.7	>250	
582	AUS1621	11.7	0.8	0.07
583	AUS1622	17.6	4.1	0.23
584	AUS1623	11.9	>250	
585	AUS1624	1.5	>250	
586	AUS1625	1.1	>250	
587	AUS1626	4.2	>250	
588	AUS1627	7.1	2.4	0.33
589	AUS1628	11.4	2.7	0.24
590	AUS1629	20.8	3.9	0.19
591	AUS1630	2.4	>250	
614	AUS1653	0.45		0.00
615	AUS1654	0.67		0.00
616	AUS1655	0.43		0.00
617	AUS1656	1.14	0.29	0.26
618	AUS1657	0.44		0.00
619	AUS1658	0.78		0.00
620	AUS1659	0.87		0.00
621	AUS1660	0.63		0.00
624	AUS1663			
627	AUS1666			
631	AUS1670	1.12	3.19	2.84
639	AUS1679	1.4	>250	
641	AUS1683	1.23	0.78	0.63
642	AUS1684	2.74	2.19	0.80
643	AUS1685	2.66	0.98	0.37

SEQ ID NO:	ID	Ratio EC ₅₀	Ratio MTT CC25 or CCK8 CC30	C/E ratio
659	AUS1705	5.7	0.3	
660	AUS1708	43.0	>250	
661	AUS1709	41.4	>250	
662	AUS1710	5.95	0.17	0.03
664	AUS1712			
665	AUS1713	5.6	3.0	0.54
666	AUS1714	8.5	3.7	0.43

Example 15: Antiviral effect of modified oligonucleotide compounds in genotype C 1.0

HBV transgenic mice

[0873] To evaluate the effect of modified oligonucleotide compounds on the serum levels of HBsAg in genotype C 1.0 HBV (HBV-GTC) transgenic mice, each compound was dosed once at 10, 30, or 40 mg/kg by subcutaneous injection (SC) to the mice and injection volume was 10 ml/kg. Normal saline was administered as negative control. The HBsAg levels was measured in the serum samples by Chemiluminescence immunoassay assays. The resulting data was expressed as mean and SD.

[0874] HBV transgenic mice, prepared from male C57BL/6 mice of 5-7 weeks old with 1.0-fold length of HBV C1 subtype genome, stably express HBsAg in liver. The methods and purposes of animal experiments are in accordance with the ethical standards and international practices.

[0875] The modified oligonucleotide dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 10 mg/ml, which was diluted with saline again at a target concentration.

[0876] The male HBV transgenic mice were randomly divided into every group according to serum HBsAg level. Each group was dosed with one compound by subcutaneous injection at a target concentration and a dose volume of 10 ml/kg of body weight or an equal volume of normal saline as control.

[0877] Approximately 80 µl of blood sample was taken from a thigh vein on Day 0 (before dosing), 3, 7, 10, 14, 21 and/or 28 after dosing. Blood samples were placed in an incubator at 37°C for approximately 30 minutes, and then centrifuged in a precooled (0-4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further HBsAg analysis.

[0878] Tables 15.1 - 15.7. (FIGS. 14-20) show the results of this experiment. Table 15.1 (FIG. 14) shows that a reduction in serum levels of HBsAg, relative to the reference compound AUS1233 was observed on Day 28 following treatment with a single 40 mg/kg subcutaneous dose of AUS1684, AUS1322, AUS1323 or AUS1324 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233. n=5 mice were tested for each group.

[0879] Table 15.2 (FIG. 15) shows that a reduction in serum levels of HBsAg, relative to the reference compound AUS1233 was observed on Day 28 following treatment with a single 40 mg/kg subcutaneous dose of AUS1169, AUS1171, AUS1170, AUS1168, or AUS1322 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233. n=4 mice were tested in each group.

[0880] Table 15.3 (FIG. 16) shows that a reduction in serum levels of HBsAg, relative to the reference compound AUS1233 was observed on Day 21 following treatment with a single 30 mg/kg subcutaneous dose of AUS1434, AUS1435, AUS1440, AUS1436, AUS1437, AUS1438, AUS1439, AUS1422, AUS1424, AUS1425, AUS1427, AUS1428, AUS1430, AUS1431, or AUS1432 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233. n=2 or 3 mice were tested in each group.

[0881] Table 15.4 (FIG. 17) shows that a reduction in serum levels of HBsAg, relative to the reference compound AUS1233 was observed on Day 21 following treatment with a single 30 mg/kg subcutaneous dose of AUS1463, AUS1466, AUS1476, AUS1472, AUS1489, or AUS1459 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233. n= 4 mice were tested in each group.

[0882] Table 15.5 (FIG. 18) shows that a reduction in serum levels of HBsAg, relative to the reference compound AUS1233 was observed on Day 28 following treatment with a single 30 mg/kg subcutaneous dose of AUS1492, AUS1495, AUS1494, or AUS1493 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233. n= 4 mice were tested in each group.

[0883] Table 15.6 (FIG. 19) shows >1.4 fold reduction in serum levels of HBsAg, relative to the reference compound AUS1233 was observed on Day 21 following treatment with a single 40 mg/kg subcutaneous dose of AUS1695 on Day 0, suggesting a persistent and

enhanced modified oligonucleotide antiviral activity relative to AUS1233. n= 1 or 2 mice were tested in each group.

[0884] Rows 1 and 2 of Table 15.7 (FIG. 20) shows a reduction in serum levels of HBsAg on Day 28 following treatment with a single 10 mg/kg subcutaneous dose of AUS1493 on Day 0 relative to treatment with a single 10mg/kg subcutaneous dose of AUS1233 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233. Rows 3 and 4 of Table 15.7 (FIG. 20) shows a reduction in serum levels of HBsAg on Day 28 following treatment with a single 30 mg/kg subcutaneous dose of AUS1493 on Day 0 relative to treatment with a single 30mg/kg subcutaneous dose of AUS1233 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

Example 16: Antiviral effect of modified oligonucleotide compounds in HDI-HBV carrier mice

[0885] To evaluate the effect of modified oligonucleotide compounds on the serum levels of HBsAg in genotype A, B, C and D (GT-A/B/C/D) hydrodynamic-injection HBV (HDI-HBV) mice, each compound was dosed once at target dosage by subcutaneous injection (SC) to the mice and injection volume was 10 ml/kg. Normal saline was administered as negative control. The HBsAg levels were measured in the serum samples by Chemiluminescence immunoassay assays. The resulting data was expressed as mean and SD.

[0886] The HDI-HBV mouse model was constructed in male C57BL/6 mice of 5-7 weeks old by hydrodynamic-injection a target concentration GT-A/B/C/D pcDNA3.1-preS2/S plasmid (or pAAV-1.2HBV plasmid) solution containing HBV sequence, HBsAg-positive mice were used 2 to 3-week after the injection. The methods and purposes of animal experiments are in accordance with the ethical standards and international practices.

[0887] The modified oligonucleotide dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 10 mg/ml, which was diluted with saline again at a target concentration.

[0888] The male GT-A/B/CD HDI-HBV mice were randomly divided into every group according to serum HBsAg level. Each group was dosed with one compound by subcutaneous injection at a target concentration and a dose volume of 10 ml/kg of body weight or an equal volume of normal saline as control.

[0889] Approximately 80 µl of blood sample was taken from a thigh vein on Day 0 (before dosing), 3, 7, 10, 14, 21, 22, and/or 28 after dosing. Blood samples were placed in an incubator at 37°C for approximately 30 minutes, and then centrifuged in a precooled (0-4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further HBsAg analysis.

[0890] Tables 16.1- 16.18. (FIG. 21-38) show the results of this experiment. Table 4.1 (FIG. 21) shows that a reduction in serum levels of HBsAg in GT-D HDI HBV mice, relative to the reference compound AUS1233 was observed on Day 28 following treatment with a single 60 mg/kg subcutaneous dose of AUS1684, AUS1322, AUS1323 or AUS1324 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

[0891] Table 16.2 (FIG. 22) shows the resulting serum levels of HBsAg in GT-D HDI HBV mice following a single 40 mg/kg subcutaneous dose of AUS1169, AUS1171, AUS1170, or AUS1168 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

[0892] Table 16.3 (FIG. 23) shows that a reduction in serum levels of HBsAg in GT-D HDI HBV mice, relative to the reference compound AUS1233 was observed on Day 21 following treatment with a single 60 mg/kg subcutaneous dose of AUS1173 or AUS1174 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

[0893] Table 16.4 (FIG. 24) shows that a reduction in serum levels of HBsAg in GT-D HDI HBV mice, relative to the reference compound AUS1233 was observed on Day 14 following treatment with a single 60 mg/kg subcutaneous dose of AUS1173, AUS1178, AUS1185, AUS1186, AUS1188, AUS1190, AUS1192, AUS1193, AUS1183, AUS1325, AUS1326, AUS1327, AUS1328, AUS1329, AUS1175, AUS1361, or AUS1362 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

[0894] Table 16.5 (FIG. 25) shows that a reduction in serum levels of HBsAg in GT-D HDI HBV mice, relative to the reference compound AUS1233 was observed on Day 22 following treatment with a single 60 mg/kg subcutaneous dose of AUS1322, AUS1382, AUS1383, AUS1384, or AUS1385 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

[0895] Table 16.6 (FIG. 26) shows the resulting serum levels of HBsAg in GT-D HDI HBV mice following a single 60 mg/kg subcutaneous dose of AUS1322, AUS1388, AUS1389, AUS1390, AUS1392, AUS1393, AUS1396, AUS1403, AUS1409, AUS1411, AUS1413, AUS1414, or AUS1415 on Day 0.

[0896] Table 16.7 (FIG. 27) shows the resulting serum levels of HBsAg in GT-D HDI HBV mice following a single 60 mg/kg subcutaneous dose of AUS1387, AUS1326, AUS1328, AUS1388, AUS1390, AUS1403, or AUS1360 on Day 0.

[0897] Table 16.8 (FIG. 28) shows that a reduction in serum levels of HBsAg in GT-D HDI HBV mice, relative to the reference compound AUS1233 was observed on Day 21 following treatment with a single 40 mg/kg subcutaneous dose of AUS1434, AUS1435, AUS1440, AUS1436, AUS1437, AUS1438, AUS1439, AUS1441, AUS1422, AUS1423, AUS1424, AUS1425, AUS1426, AUS1427, AUS1428, AUS1429, AUS1430, AUS1431, AUS1432, or AUS1433 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

[0898] Table 16.9 (FIG. 29) shows that a reduction in serum levels of HBsAg in GT-D HDI HBV mice, relative to the reference compound AUS1233 was observed on Day 21 following treatment with a single 40 mg/kg subcutaneous dose of AUS1444, AUS1458, AUS1459, AUS1460, AUS1461, AUS1462, AUS1463, AUS1464, AUS1465, AUS1466, AUS1467, AUS1468, AUS1469, AUS1470, AUS1471, AUS1472, AUS1473, AUS1474, AUS1476, AUS1477, AUS1478, AUS1479, AUS1480, AUS1481, AUS1482, AUS1488, AUS1489, AUS1490, AUS1443, AUS1444, or AUS1445 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

[0899] Table 16.10 (FIG. 30) shows that a reduction in serum levels of HBsAg in GT-D HDI HBV mice, relative to the reference compound AUS1233 was observed on Day 28 following treatment with a single 15 mg/kg subcutaneous dose of AUS1492 or AUS1493 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

[0900] Rows 1, 2 and 3 of Table 4.11 (FIG. 31) shows a reduction in serum levels of HBsAg in GT-A HDI HBV mice, observed on Day 28 following treatment with a single 15 mg/kg subcutaneous dose of AUS1492 or AUS1493 on Day 0, relative to a single 15 mg/kg subcutaneous dose of AUS1233 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

[0901] Rows 3, 4 and 5 of Table 4.11 (FIG. 31) shows a reduction in serum levels of HBsAg in GT-A HDI HBV mice, observed on Day 28 following treatment with a single 45 mg/kg subcutaneous dose of AUS1492 or AUS1493 on Day 0, relative to a single 45 mg/kg subcutaneous dose of AUS1233 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

[0902] Table 4.12 (FIG. 32) shows the resulting serum levels of HBsAg in GT-A HDI HBV mice following a single 15 or 45 mg/kg subcutaneous dose of AUS1441, AUS1466, AUS1472, AUS1488, or AUS1489 on Day 0.

[0903] Table 4.13 (FIG. 33) shows the resulting serum levels of HBsAg in GT-D HDI HBV mice following a single 15 or 45 mg/kg subcutaneous dose of AUS1495, AUS1494, AUS1492, or AUS1441 on Day 0.

[0904] Table 4.14 (FIG. 34) shows the resulting serum levels of HBsAg in GT-A HDI HBV mice following a single 15 or 45 mg/kg subcutaneous dose of AUS1495, AUS1494, AUS1492, AUS1482, or AUS1441 on Day 0.

[0905] Table 4.15 (FIG. 35) shows that a reduction in serum levels of HBsAg in GT-B HDI HBV mice, relative to the reference compound AUS1233 was observed on Day 21 following treatment with a single 40 mg/kg subcutaneous dose of AUS1684 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

[0906] Table 4.16 (FIG. 36) shows that a reduction in serum levels of HBsAg in GT-A 1.2HBV transgenic mice, relative to the reference compound AUS1233 was observed on Day 28 following treatment with a single 45 mg/kg subcutaneous dose of AUS1492 or AUS1493 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

[0907] Table 4.17 (FIG. 37) shows that a reduction in serum levels of HBsAg in GT-A HDI HBV mice, relative to the reference compound AUS1233 was observed on Day 21 following treatment with a single 40 mg/kg subcutaneous dose of AUS1396, AUS1422, AUS1423, AUS1424, AUS1426, AUS1427, AUS1428, AUS1429, AUS1430, AUS1431, AUS1432, or AUS1433 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

[0908] Table 4.18 (FIG. 38) shows that a reduction in serum levels of HBsAg in GT-A HDI HBV mice, relative to the reference compound AUS1233 was observed on Day 21

following treatment with a single 40 mg/kg subcutaneous dose of AUS1444, AUS1458, AUS1459, AUS1460, AUS1461, AUS1462, AUS1463, AUS1464, AUS1465, AUS1466, AUS1467, AUS1468, AUS1470, AUS1471, AUS1472, AUS1473, AUS1475, AUS1476, AUS1478, AUS1479, AUS1480, AUS1481, AUS1483, AUS1489, AUS1490, AUS1443, or AUS1434 on Day 0, suggesting a persistent and enhanced modified oligonucleotide antiviral activity relative to AUS1233.

Example 17: Antiviral effect of eASO compounds in AAV-HBV carrier mice

[0909] To evaluate effect of eASO compounds on the serum levels of HBsAg in adeno-associated virus-hepatitis B virus (AAV-HBV) transfected mice, each compound was dosed once at target dosage by subcutaneous injection (SC) to the mice and injection volume was 10 ml/kg. Normal saline was administered as negative control.

[0910] The male C57BL/6 mice were aged 4-5 weeks and prepared, all the mice were injected through tail vein with 1×10^{11} vector genome of AAV-HBV in 200 μ L phosphate buffered saline per mouse on predose Day 0. Animal clinical signs were monitored twice daily during model development phase, and body weight was measured on predose Days 0, 21, and 28. Serum samples of per mouse prepared on Predose Day 21, 28 were transferred to the quantitative detections of HBsAg, HBeAg, and HBV DNA levels, HBsAg-positive mice were used 4 to 5 week after the injection. The methods and purposes of these animal experiments are in accordance with the ethical standards and international practices.

[0911] The eASO dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 10 mg/ml, which was diluted with saline again at a target concentration.

[0912] Based on animal body weight and serum HBsAg, HBeAg, HBV DNA levels on predose Day 28, all transfected mice were randomized into each group. Each group was dosed with one compound by subcutaneous injection at a target concentration and a dose volume of 10 ml/kg of body weight or an equal volume of normal saline as control.

[0913] Approximately 80 μ L of blood sample was taken from a thigh vein on Day 0 (before dosing), 3, 7, 10, 14, 21 and 28 after dosing. Blood samples were placed in an incubator at 37°C for approximately 30 minutes, and then centrifuged in a precooled (0-4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further HBsAg, HBeAg, HBV DNA analysis. Serum HBsAg and HBeAg levels were detected using ARCHITECT i2000 (Abbott Laboratories, Lake Bluff, IL, USA)

with supporting reagents. Serum HBV DNA levels were analyzed by QuantStudio™ 3 System (Applied Biosystems, Foster City, CA, USA) with detection kit (Sansure Biotech Inc., Changsha, Hunan, China).

[0914] Table 17 (FIG. 39) show the results of this experiment, a reduction in serum levels of HBsAg/HBeAg/HBV DNA in GT-D AAV HBV mice, relative to the reference compound AUS1233 was observed on Day 28 following treatment with a single 40 mg/kg subcutaneous dose of AUS1493 on Day 0, suggesting a persistent and enhanced eASO antiviral activity relative to AUS1233.

Example 18: Toxicity evaluation of eASO compounds in male C57BL/6 mice

[0915] To evaluate effect of eASO compounds on the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in male C57BL/6 mice, each compound was dosed triple at target dosage by subcutaneous injection (SC) on Day 0, 2, 4 and injection volume was 10 ml/kg. Normal saline was administered as negative control.

[0916] The male C57BL/6 mice were age d 6-7 weeks and prepared. The eASO dosing solutions were prepared by dissolving each compound in saline to make a stock solution at 20 mg/ml, which was diluted with saline again at a target concentration.

[0917] Based on animal body weight, all mice were randomized into each group. Each group was dosed with one compound by subcutaneous injection at a target concentration and a dose volume of 10 ml/kg of body weight or an equal volume of normal saline as control.

[0918] Approximately 80 µl of blood sample was taken from a thigh vein on Day 7, 10, 14, 21 and 28 after dosing. Blood samples were placed in an incubator at 37°C for approximately 30 minutes, and then centrifuged in a precooled (0-4°C) centrifuge to obtain the serum samples. The obtained serum samples were stored in a -20°C refrigerator for further ALT and analysis. Serum ALT and AST levels were detected using colorimetric analysis kits.

[0919] Tables 18.1-18.2 (FIG. 40-41) show the results of this experiment. The tables shows that a reduction in serum levels of ALT in WT male C57BL/6 mice, relative to the reference compound AUS1233 when the mice were dosed on Day 0, 2 and 4 at 60 mg/kg by SC (n=3 mice for every group). A reduction of serum ALT levels were observed on Day 21 following treatment with AUS1434, AUS1435, AUS1440, AUS1436, AUS1437, AUS1438, AUS1439, AUS1441, AUS1178, AUS1190, AUS1188, AUS1192, AUS1422, AUS1423, AUS1424, AUS1425, AUS1426, AUS1427, AUS1428, AUS1429, AUS1430, AUS1431,

AUS1432, AUS1433, AUS1360, AUS1401, AUS1361, AUS1362, AUS1443, AUS1444, AUS1445, AUS1446, AUS1447, AUS1448, AUS1449, AUS1450, AUS1452, AUS1453, AUS1454, AUS1455, AUS1456, or AUS1457, suggesting a reduced eASO toxicity relative to AUS1233 over time. These results show that the modified oligonucleotides can result in a 1.2 fold to 7.7 fold reduction in serum ALT levels on Day 21, suggesting reduced toxicity.

CLAIMS**What is claimed is:**

1. A compound comprising a modified oligonucleotide, the modified oligonucleotide comprising from 5' to 3':

5' W1 - G1 - S1 - G2 - W2 3' (Formula I)

wherein:

W1 is a 5' wing segment;

W2 is a 3' wing segment;

G1 is a first gap segment;

S1 is a first separator segment;

G2 is a second gap segment;

- is an internucleoside linkage; and

at least one nucleoside of the oligonucleotide is modified.

2. The compound of claim 1, wherein the modified oligonucleotide comprises from 5' to 3':

5' W1 - G1 - S1 - G2 - S2 - G3 - W2 3' (Formula II)

wherein:

S2 is a second separator segment; and

G3 is a third gap segment.

3. The compound of claim 2, wherein the modified oligonucleotide comprises from 5' to 3':

5' W1 - G1 - S1 - G2 - S2 - G3 - S3 - G4 - W2 3' (Formula III)

wherein:

S3 is a third separator segment; and

G4 is a fourth gap segment.

4. The compound of claim 3, wherein the modified oligonucleotide comprises from 5' to 3':

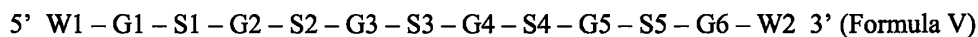
5' W1 - G1 - S1 - G2 - S2 - G3 - S3 - G4 - S4 - G5 - W2 3' (Formula IV)

wherein:

S4 is a fourth separator segment; and

G5 is a fifth gap segment.

5. The compound of claim 4, wherein the modified oligonucleotide comprises from 5' to 3':



wherein:

S5 is a fifth separator segment; and

G6 is a sixth gap segment.

6. The compound of claim 5, wherein the modified oligonucleotide comprises from 5' to 3':



(Formula VI)

wherein:

S6 is a sixth separator segment; and

G7 is a seventh gap segment.

7. The compound of any one of claims 1 to 6, wherein W1 comprises one or more linked deoxynucleosides.

8. The compound of any one of claims 1 to 7, wherein W2 comprises one or more linked deoxynucleosides.

9. The compound of any one of claims 1 to 8, wherein W1 comprises 2 to 25 linked nucleosides.

10. The compound of any one of claims 1 to 9, wherein W2 comprises 2 to 35 linked nucleosides.

11. The compound of any one of claims 1 to 10, wherein G1 comprises 1 to 10 linked deoxynucleosides.

12. The compound of any one of claims 1 to 11, wherein G2 comprises 1 to 10 linked deoxynucleosides.

13. The compound of any one of claims 1 to 12, wherein S1 comprises 1 to 3 linked nucleosides.

14. The compound of any one of claims 2 to 13, wherein G3 comprises 1 to 10 linked deoxynucleosides.

15. The compound of any one of claims 2 to 14, wherein S2 comprises 1 to 3 linked nucleosides.
16. The compound of any one of claims 3 to 15, wherein G4 comprises 1 to 10 linked deoxynucleosides.
17. The compound of any one of claims 3 to 16, wherein S3 comprises 1 or 2 linked nucleosides.
18. The compound of any one of claims 4 to 17, wherein G5 comprises 1 to 10 linked deoxynucleosides.
19. The compound of any one of claims 4 to 17, wherein S4 comprises 1 to 3 linked nucleosides.
20. The compound of any one of claims 5 to 19, wherein G6 comprises 1 to 10 linked deoxynucleosides.
21. The compound of any one of claims 5 to 19, wherein S5 comprises 1 to 3 linked nucleosides.
22. The compound of any one of claims 6 to 21, wherein G7 comprises 1 to 10 linked deoxynucleosides.
23. The compound of any one of claims 6 to 22, wherein S6 comprises 1 to 3 linked nucleosides.
24. The compound of any one of claims 1 to 23, wherein the oligonucleotide is 18-50 nucleobases in length.
25. The compound of any one of claims 1 to 23, wherein the oligonucleotide is at least 20 nucleobases in length.
26. The compound of any one of claims 1 to 23, wherein the oligonucleotide is 20 nucleobases in length.

27. The compound of claim 1, wherein the oligonucleotide is 20 linked nucleobases in length.
28. The compound of claim 27, wherein W1 comprises 4 linked nucleosides.
29. The compound of claim 27, wherein W1 comprises 5 linked nucleosides.
30. The compound of any one of claims 27 to 29, wherein G1 comprises 4 linked deoxynucleosides.
31. The compound of any one of claims 27 to 29, wherein G1 comprises 5 linked deoxynucleosides.
32. The compound of any one of claims 27 to 29, wherein G1 comprises 6 linked deoxynucleosides.
33. The compound of any one of claims 27 to 32, wherein G2 comprises 4 linked deoxynucleosides.
34. The compound of any one of claims 27 to 32, wherein G2 comprises 5 linked deoxynucleosides.
35. The compound of any one of claims 27 to 32, wherein G2 comprises 6 linked deoxynucleosides.
36. The compound of any one of claims 27 to 33, wherein S1 comprises at least one linked nucleoside.
37. The compound of any one of claims 27 to 35, wherein W2 comprises 5 linked nucleosides.
38. The compound of any one of claims 27 to 35, wherein W2 comprises 6 linked nucleosides.
39. The compound of any one of claims 27 to 38, wherein the S1 is at position 10 of the oligonucleotide.

40. The compound of claim 39, wherein W1 comprises 4 linked nucleosides, G1 comprises 5 linked deoxynucleosides, S1 comprises 1 linked nucleoside, G2 comprises 5 linked deoxynucleosides, and W2 comprises 5 linked nucleosides.
41. The compound of claim 1, wherein W1 comprises 4-6 linked nucleosides.
42. The compound of claim 41, wherein W2 comprises 4-6 linked nucleosides.
43. The compound of any one of claims 41 to 42, wherein G1 comprises 1-6 linked deoxynucleosides.
44. The compound of any one of claims 41 to 43, wherein G2 comprises 1-6 linked deoxynucleosides.
45. The compound of any one of claims 41 to 44, wherein G3 comprises 1-6 linked deoxynucleosides.
46. The compound of any one of claims 41 to 45, wherein S1 comprises least one linked nucleoside.
47. The compound of any one of claims 41 to 46, wherein the length of the G1-S1-G2 is between 8 and 12 nucleobases in length.
48. The compound of any one of claims 1 to 47, wherein any one or more of G1, G2, G3, G4, G5, G6, and G7 comprises a nucleoside comprising a modification to a 2'-deoxynucleoside.
49. The compound of claim 48, wherein any one or more of G1, G2, G3, G4, G5, G6, and G7 comprises a nucleoside comprising a 2'-deoxy 5-methylcytidine sugar modification.
50. The compound of any one of claims 1 to 49, wherein S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-O-methoxyethyl sugar modification.
51. The compound of any one of claims 1 to 49, wherein S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 5-methylcytidine.

- 52.** The compound of any one of claims 1 to 49, wherein S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-O-methyl sugar modification.
- 53.** The compound of any one of claims 1 to 52, wherein S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-OH sugar modification.
- 54.** The compound of any one of claims 1 to 53, wherein S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-fluoro sugar modification.
- 55.** The compound of any one of claims 1 to 54, wherein S1, S2, S3, S4, S5, and/or S6 comprises a nucleoside comprising a 2'-fluoro-arabinonucleic acid (2'-fluoro-ANA) sugar modification.
- 56.** The compound of any one of claims 1 to 55, wherein S1, S2, S3, S4, S5, and/or S6 comprises a glycol nucleic acid (GNA).
- 57.** The compound of any one of claims 1 to 56, wherein S1, S2, S3, S4, S5, and/or S6 comprises a LNA.
- 58.** The compound of any one of claims 1 to 57, wherein W1 comprises a nucleoside comprising a 2'-deoxy sugar modification.
- 59.** The compound of claim 58, wherein the 2'-deoxy sugar modification is at positions 2 and/or 5 of a sequence corresponding to SEQ ID NO: 2.
- 60.** The compound of any one of claims 1 to 59, wherein W1 comprises a nucleoside comprising a 2'-O-methoxyethyl sugar modification.
- 61.** The compound of claim 60, wherein the 2'-O-methoxyethyl sugar modification is at position 1, 2, 3, 4 and/or 5 of a sequence corresponding to SEQ ID NO: 2.
- 62.** The compound of any one of claims 1 to 61, wherein W1 comprises a 2'-O-methoxyethyl 5-methylcytidine at position 2 of a sequence corresponding to SEQ ID NO: 2.
- 63.** The compound of any one of claims 1 to 62, wherein W1 comprises a nucleoside comprising a 2'-O-methyl sugar modification.

64. The compound of claim 63, wherein the 2'-O-methyl sugar modification is at positions 1, 2, 3, 4 and/or 5 of a sequence corresponding to SEQ ID NO: 2.
65. The compound of claim 63, wherein W1 comprises a 2'-O-methyl 5-methylcytidine at position 2 of a sequence corresponding to SEQ ID NO: 2.
66. The compound of any one of claims 1 to 65, wherein W1 comprises a nucleoside comprising a 2'-fluoro sugar modification.
67. The compound of any one of claims 1 to 66, wherein W1 comprises a nucleoside comprising a 2'-fluoro-arabinonucleic acid (2'-fluoro-ANA) modification.
68. The compound of any one of claims 1 to 67, wherein W1 comprises a glycol nucleic acid (GNA).
69. The compound of any one of claims 1 to 67, wherein the GNA is at position 2 of a sequence corresponding to SEQ ID NO: 2.
70. The compound of any one of claims 1 to 69, wherein W1 comprises a modified nucleoside and wherein the modified nucleoside is a locked nucleic acid (LNA).
71. The compound of claim 70, wherein the LNA is at positions 1 and/or 3 of a sequence corresponding to SEQ ID NO: 2.
72. The compound of any one of claims 1 to 71, wherein W2 comprises a nucleoside comprising a 2'-deoxy sugar modification.
73. The compound of claim 72, wherein the 2'-deoxy sugar modification is at positions 15 and/or 16 of a sequence corresponding to SEQ ID NO: 2.
74. The compound of any one of claims 1 to 73, wherein W2 comprises a nucleoside comprising a 2'-O-methoxyethyl sugar modification.
75. The compound of claim 74, wherein the 2'-O-methoxyethyl sugar modification is at positions 15, 16, 17, 18, 19 and/or 20 of a sequence corresponding to SEQ ID NO: 2.

76. The compound of claim 74, wherein W2 comprises a 2'-O-methoxyethyl 5-methylcytidine at position 20 of a sequence corresponding to SEQ ID NO: 2.
77. The compound of any one of claims 1 to 76, wherein W2 comprises a nucleoside comprising a 2'-O-methyl sugar modification.
78. The compound of claim 77, wherein the 2'-O-methyl sugar modification is at positions 15, 16, 17, 18, 19 and/or 20 of a sequence corresponding to SEQ ID NO: 2.
79. The compound of claim 77, wherein W2 comprises a 2'-O-methyl 5-methylcytidine at position 20 of a sequence corresponding to SEQ ID NO: 2.
80. The compound of any one of claims 1 to 79, wherein W2 comprises a nucleoside comprising a 2'-fluoro sugar modification.
81. The compound of any one of claims 1 to 80, wherein W2 comprises a nucleoside comprising a 2'-fluoro-arabinonucleic acid (2'-fluoro-ANA) modification.
82. The compound of any one of claims 1 to 81, wherein W2 comprises a modified nucleoside and wherein the modified nucleoside is a glycol nucleic acid (GNA).
83. The compound of any one of claims 1 to 82, wherein W2 comprises a modified nucleoside and wherein the modified nucleoside is a locked nucleic acid (LNA).
84. The compound of claim 83, wherein the LNA is at positions 16, 17, 18, 19 and/or 20 of a sequence corresponding to SEQ ID NO: 2.
85. The compound of any one of claims 1 to 84, wherein at least one internucleoside linkage is a phosphodiester linkage.
86. The compound of any one of claims 1 to 85, wherein at least one internucleoside linkage is a modified internucleoside linkage.
87. The compound of claim 86, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

- 88.** The compound of any one of claims 1 to 86, wherein the oligonucleotide is complementary to a portion of the polynucleotide sequence of a HBV genome.
- 89.** The compound of claim 88, wherein the HBV genome is the genome of HBV GT-A, GT-B, GT-C, GT-D, GT-E, GT-F, GT-G, or GT-H.
- 90.** The compound of claim 88, wherein the oligonucleotide is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% complementary to a portion of the polynucleotide sequence of any one of SEQ ID NOS: SEQ ID NO 3, and 667-674.
- 91.** The compound of claim 88, wherein the oligonucleotide is complementary to the polynucleotide sequence of any one of SEQ ID NOS: 1, 4, and 675-838.
- 92.** The compound of any one of claims 1 to 91, wherein the oligonucleotide comprises a nucleobase sequence of a sequence corresponding to SEQ ID NO: 2.
- 93.** A compound comprising the modified oligonucleotide of any one of SEQ ID NOS: 11-666, or a oligonucleotide comprising at least 1, 2, 3, 4, or 5 further modifications thereto.
- 94.** A compound comprising the modified oligonucleotide of any one of SEQ ID NOS: 11-666, or a oligonucleotide having at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% sequence identity thereto.
- 95.** A pharmaceutical composition comprising the compound of any one of claims 1 to 94, or salt thereof and at least one pharmaceutically acceptable carrier or diluent.
- 96.** A formulation comprising about 100 mg/mL, 150 mg/mL, or 200 mg/mL of a compound comprising the modified oligonucleotide sequence of SEQ ID NO: 456, or a oligonucleotide comprising at least 1, 2, 3, 4, or 5 further modifications thereto.
- 97.** A method of reducing HBV antigen levels in a subject comprising administering to the subject the compound of any one of claims 1 to 94, the composition of claim 95 or the formulation of claims 96.

- 98.** The method of claim 97, wherein the HBV antigen is HBsAg or HBeAg.
- 99.** The method of claim 97, wherein HBeAg levels are reduced.
- 100.** The method of claim 97, wherein HBsAg levels are reduced.
- 101.** The method of claim 97, wherein, wherein the HBsAg levels are reduced by at least about 1.25 fold relative to a subject administered with a composition comprising the modified oligonucleotide sequence of SEQ ID NO: 10.
- 102.** The method of claim 97, wherein the HBsAg levels are reduced by about 2 fold to about 10 fold relative to a subject administered with a composition comprising the modified oligonucleotide sequence of SEQ ID NO: 10.
- 103.** A method of reducing liver toxicity and/or persistence in a subject having HBV comprising administering to a subject the compound of any one of claims 1 to 94, the composition of claim 95 or the formulation of claims 96.
- 104.** The method of claim 103, wherein ALT, ALP, and/or AST levels are reduced, optionally by at least about 1.25 fold relative to a subject administered with a composition comprising the modified oligonucleotide sequence of SEQ ID NO: 10.
- 105.** A method of preventing, treating, ameliorating or slowing the progression of a HBV-related disease, disorder or condition comprising administering to a subject the compound of any one of claims 1 to 94, the composition of claim 95 or the formulation of claims 96.
- 106.** The method of any one of claims 97 to 105, wherein the subject is administered with at least one, at least 2, at least 3, at least 4 or at least 5 doses of the compound, the composition or the formulation.
- 107.** The method of any one of claims 97 to 106, wherein the subject is dosed on Day 1, Day 4 and Day 8.
- 108.** The method of claim 107, wherein the subject is dosed once every week after Day 8.

109. The method of any one of claims 97 to 108, wherein the subject is dosed on at least Day 1, Day 4 and Day 8, Day 15 and Day 22.
110. The method of any one of claims 107 to 109, wherein the amount of each dose is substantially the same amount.
111. The method of any one of claims 97 to 110, wherein the dose is about 30 mg to about 600 mg.
112. The method of any one of claims 97 to 110, wherein the dose is about 100 mg to about 500 mg.
113. The method of any one of claims 97 to 110, wherein the dose is about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 450 mg, or about 500 mg.
114. The method of any one of claims 97 to 113, wherein the administering to a subject is parenteral.
115. The method of any one of claims 97 to 113, wherein the administering to a subject is subcutaneous.
116. The method of any one of claims 97 to 113, wherein the administering to a subject is transdermal.
117. The method of any one of claims 97 to 113, wherein the administering to a subject is intraocular.
118. The method of any one of claims 97 to 113, wherein the administering to a subject is intramuscular.
119. The method of any one of claims 97 to 113, wherein the administering to a subject is intravenous.
120. The method of any one of claims 97 to 115, wherein the method further comprises administering at least a second agent to the subject.

- 121.** The method of any one of claims 97 to 120, wherein the disease, disorder or condition is a liver disease, disorder or condition.
- 122.** The method of claim 121, 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 infection, HBV viremia, or liver disease-related transplantation.
- 123.** The method of claim 121, wherein the disease or condition is a hyperproliferative condition.
- 124.** The method of claim 123, wherein the hyperproliferative condition is liver cancer.
- 125.** The method of any one of claims 97 to 120, wherein the disease, disorder or condition is a hepatitis delta virus infection (HDV), wherein the subject is co-infected with HBV.
- 126.** The method of any one of claims 97 to 125, wherein the subject is human.