(12) STANDARD PATENT (11) Application No. **AU 2017326372 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Modified oligonucleotides and methods of use

(51) International Patent Classification(s)
C12N 15/113 (2010.01) ***A61K 31/7125*** (2006.01)
A61K 31/712 (2006.01) *C07H 21/02* (2006.01)

(21) Application No: **2017326372** (22) Date of Filing: **2017.09.14**

(87) WIPO No: **WO18/053185**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/394,739	**2016.09.14**	**US**
62/394,738	**2016.09.14**	**US**

(43) Publication Date: **2018.03.22**
(44) Accepted Journal Date: **2023.12.07**

(71) Applicant(s)
Janssen Biopharma, Inc.

(72) Inventor(s)
Gryaznov, Sergei;Beigelman, Leonid;Hong, Jin;Rajwanshi, Vivek;Martínez-Monero, Saúl

(74) Agent / Attorney
Spruson & Ferguson, GPO Box 3898, Sydney, NSW, 2001, AU

(56) Related Art
WO 2016/077321 A1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 March 2018 (22.03.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/053185 A1

(51) International Patent Classification:
C12N 15/113 (2010.01) *A61K 31/7125* (2006.01)
A61K 31/712 (2006.01) *C07H 21/02* (2006.01)

(21) International Application Number:
PCT/US2017/051644

(22) International Filing Date:
14 September 2017 (14.09.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/394,738 14 September 2016 (14.09.2016) US
62/394,739 14 September 2016 (14.09.2016) US

(71) Applicant: **ALIOS BIOPHARMA, INC.** [US/US]; 260 East Grand Avenue, 2nd Floor, South San Francisco, California 94080 (US).

(72) Inventors: **GRYAZNOV, Sergei**; 260 East Grand Avenue, 2nd Floor, South San Francisco, California 94080 (US). **BEIGELMAN, Leonid**; 260 East Grand Avenue, 2nd Floor, South San Francisco, California 94080 (US). **HONG, Jin**; 260 East Grand Avenue, 2nd Floor, South San Francisco, California 94080 (US). **RAJWANSHI, Vivek**; 260 East Grand Avenue, 2nd Floor, South San Francisco, California 94080 (US). **MARTINEZ-MONERO, Saul**; 260 East Grand Avenue, 2nd Floor, South San Francisco, California 94080 (US).

(74) Agent: **SHIRTZ, Joseph F.** et al.; Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, New Jersey 08933 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

(54) Title: MODIFIED OLIGONUCLEOTIDES AND METHODS OF USE

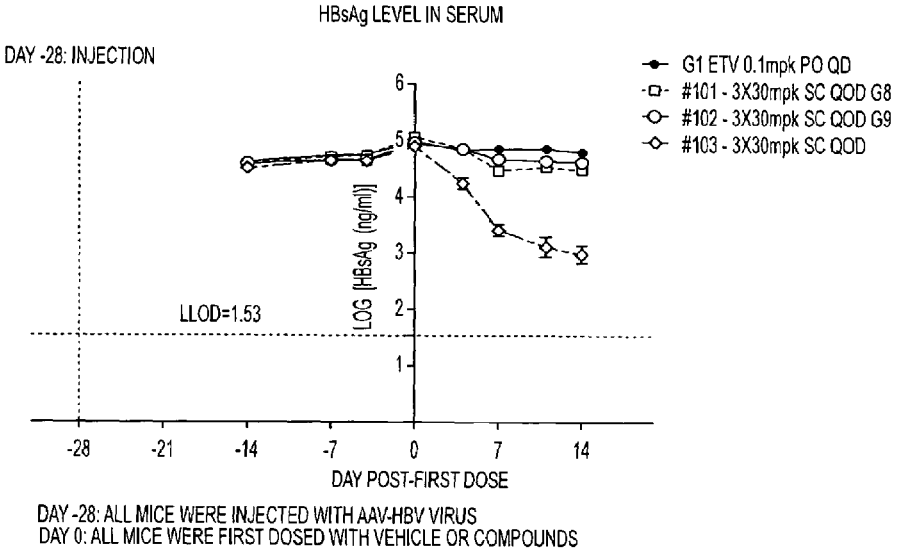


FIG. 1A

(57) Abstract: Modified oligonucleotides comprising modifications at the 2' and/or 3' positions(s) along with methods of making and use, e.g., against HBV are disclosed.

[Continued on next page]

WO 2018/053185 A1

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

MODIFIED OLIGONUCLEOTIDES AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a U.S. application claiming the benefit of priority to U.S Provisional Application No. 62/394,738, filed September 14, 2016 and U.S Provisional Application No. 62/394,739, filed September 14, 2016, the entireties of which are hereby incorporated by reference.

BACKGROUND

[0002] Antisense oligonucleotide therapies have been considered for treatment or prevention of various diseases and conditions such as viral diseases, neurological diseases, neurodegenerative diseases, fibrotic diseases, hyperproliferative diseases.

[0003] Certain viral diseases such as hepatitis B (HBV) remain elusive from conventional therapies while continuing to infect an estimated 240 million people (defined as HBV surface antigen positive for at least 6 months) and contributing to the deaths of more than 686,000 people every year. Conventional therapies including oral anti-viral nucleotide analog treatments, such as tenofovir or entecavir, only suppresses the replication of the virus and do not cure the HBV infection. Therefore, even those treated with current HBV therapies must continue their treatment for life.

[0004] Oligonucleotides can bind a complimentary RNA or DNA sequence. This feature enables oligonucleotides to bind specific nucleic acid targets involved in many aspects of cellular processes such as metabolism, differentiation, proliferation, viral replication, etc. Oligonucleotides can also be engineered to cleave target RNA through RNase H mechanism or RISC pathway; block micro RNA binding, change RNA splicing pattern, or bind to targets as aptamers once they bind to their specific target. For example, chimeric oligonucleotides, such as "gapmers" include a portion of the oligonucleotide that attracts RNase H enzyme to the site where the oligonucleotide binds to the RNA region. Subsequent activation of RNase H results in cleavage of the genetic target, thereby inhibiting the function of the genetic target such as gene expression or replication of a virus.

[0005] Accordingly, there is a need in the art to discover and develop new therapies with different mechanisms of action, increased potency, increased affinity and/or decreased side-effects.

[0005a] Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

[0005b] Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise”, “comprising”, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”.

SUMMARY

[0006] The present disclosure relates to compounds and compositions containing oligonucleotides and their use in preventing or treating diseases and conditions, e.g., HBV.

[0006a] In one aspect, the present disclosure provides a chimeric antisense oligonucleotide represented by Formula (A):

5' X—Y—Z 3' (A)

wherein:

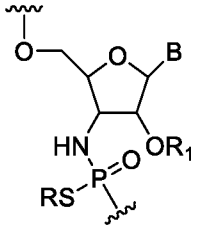
X—Y—Z is a chimeric oligonucleotide comprising a sequence of 18 to 22 nucleosides, optionally conjugated at the 5' and/or 3' end to a ligand targeting group;

X is a domain comprising a sequence of modified nucleosides that is 3-10 nucleosides in length;

Z is a domain comprising a sequence of modified nucleosides that is 3-10 nucleosides in length;

Y is a domain comprising a sequence of 2 to 10 2’-deoxy-nucleosides linked through thiophosphate intersubunit linkages;

each modified nucleoside in the X domain and each modified nucleoside in the Z domain are nucleosides of Formula (1)



(1);

R is H or a positively charged counter ion;

B is a nucleobase;

R_1 is $-(CR'_2)_2OCR'_3$ or –OEt;

R' is independently in each instance H or F; and

said oligonucleotide is complementary to a sequence of the HBV genome.

[0006b] In another aspect, the present disclosure provides a chimeric antisense oligonucleotide represented by Formula (A):

5' X-Y-Z 3' (A)

wherein:

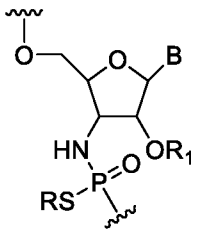
X—Y—Z is a chimeric oligonucleotide comprising a sequence of 18 to 22 nucleosides, optionally conjugated at the 5' and/or 3' end to a ligand targeting group;

X is a domain comprising a sequence of modified nucleosides that is 3-10 nucleosides in length;

Z is a domain comprising a sequence of modified nucleosides that is 3-10 nucleosides in length;

Y is a domain comprising a sequence of 2 to 10 2'-deoxy-nucleosides linked through thiophosphate intersubunit linkages;

each modified nucleoside in the X domain and each modified nucleoside in the Z domain are nucleosides of Formula (1)



(1);

R is H or a positively charged counter ion;

B is a nucleobase;

R_1 is $–CR'_3$, $–CR'_2OCR'_3$, $–(CR'_2)_3OCR'_3$ or $–(CR'_2)_{1-2}CR'_3$,$–(CR'_2)_2OCR'_3$ or –Et;

R' is independently in each instance H or F; and

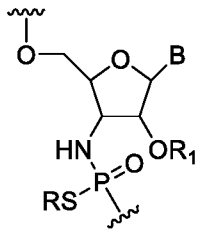
the chimeric antisense oligonucleotide comprises a nucleobase sequence that is complementary or hybridizes to a target RNA.

[0006c] In another aspect, the present disclosure provides a pharmaceutical composition comprising an oligonucleotide of the invention and a pharmaceutically acceptable excipient.

[0006d] In another aspect, the present disclosure provides a method of treating a subject having a viral infection, comprising administering to the subject a therapeutically effective amount of an oligonucleotide of the invention of a pharmaceutical composition of the invention.

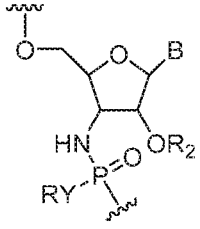
[0006e] In another aspect, the present disclosure provides use of an oligonucleotide of the invention of a pharmaceutical composition of the invention in the manufacture of a medicament for the treatment of a subject having a viral infection.

[0007] Some embodiments include an oligonucleotide comprising one or more nucleotides of Formula (I):



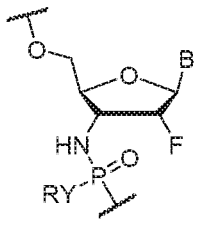
(I), wherein R is H or a positively charged counter ion, B is a nucleobase, R_1 is $–(CR'_2)_2OCR'_3$, and R' is independently in each instance H or F. In some embodiments, each nucleotide of said oligonucleotide is a nucleotide of Formula (I). In some embodiments, the oligonucleotide comprises 2 to 40 nucleotides. In some embodiments, the oligonucleotide comprises 2-26 nucleotides of Formula (I). In some embodiments, the oligonucleotide comprises 5-10 nucleotides of Formula (I). In some embodiments, B is an unmodified nucleobase in at least one nucleotide of Formula (I). In some embodiments, B is a modified nucleobase in at least one nucleotide of Formula (I). In some embodiments, B is an unmodified nucleobase in each nucleotide of Formula (I). In some embodiments, B is a modified nucleobase in each nucleotide of Formula (I). In some embodiments, each R' is H in at least one nucleotide of Formula (I). In some embodiments, each R' is H in each nucleotide of Formula (I). In some embodiments, R_1 is

–$(CH_2)_2OCH_3$ in at least one nucleotide of Formula (I). In some embodiments, R_1 is –$(CH_2)_2OCH_3$ in each nucleotide of Formula (I). In some embodiments, the oligonucleotide further comprises one or more nucleotides of Formula (II):

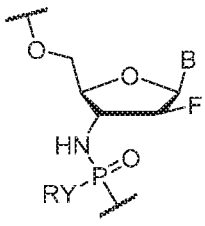


(II),

wherein Y is S or O, R is H or a positively charged counter ion, B is a nucleobase, R_2 is $-CR'_3$, $-CR'_2OCR'_3$, $-(CR'_2)_3OCR'_3$ or $-(CR'_2)_{1-2}CR'_3$, or R_2 is $-(CR'_2)_2OCR'_3$ and Y is O, and R' is independently in each instance H or F. In some embodiments, the oligonucleotide comprises at least one nucleotide of Formula (II), where R_2 is $-CR'_3$. In some embodiments, the oligonucleotide comprises at least one nucleotide of Formula (II), where R_2 is $-(CR'_2)_{1-2}OCR'_3$. In some embodiments, the oligonucleotide comprises at least one nucleotide of Formula (II), where R_2 is $-(CR'_2)_{1-2}CR'_3$. In some embodiments, B is a modified nucleobase in at least one nucleotide of Formula (II). In some embodiments, Y is S in at least one nucleotide of Formula (II). In some embodiments, Y is O in at least one nucleotide of Formula (II). In some embodiments, Y is S in each nucleotide of Formula (II). In some embodiments, Y is O in each nucleotide of Formula (II). In some embodiments, the oligonucleotide further comprises one or more nucleotides of Formula (IIIa) or Formula (IIIb):

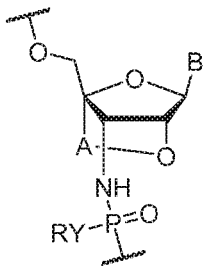


(IIIa)



(IIIb),

wherein Y is S or O, R is H or a positively charged counter ion, and B is a nucleobase. In some embodiments, the oligonucleotide further comprises one or more nucleotides of Formula (V'):



(V'),

wherein Y is S or O, R is H or a positively charged counter ion, B is independently in each instance a natural or an unmodified nucleobase or a modified nucleobase, A is –(CR''R'')$_{1-2}$– and R'' is independently in each instance H, F or Me. In some embodiments, the oligonucleotide is arranged in a construct of Formula (VI): 5' X—Y—Z 3' (VI), wherein each of X, Y and Z is a domain comprising 2-14 nucleotides, at least one of the X and Z domains comprising at least one nucleotide of Formula (I), and wherein each of the nucleotides of the Y domain is a 2'-deoxynucleotide. In some embodiments, the oligonucleotide comprises 18 to 22 nucleosides. In some embodiments, the X and Z domains each comprise 5-10 nucleotides. In some embodiments, the Y domain comprises 5-10 nucleotides. In some embodiments, the X and Z domains each comprise 5-10 nucleotides, and the Y domain comprises 5-10 nucleotides. In some embodiments, the X and Z domains each comprise 5 nucleotides, and the Y domain comprises 10 nucleotides. In some embodiments, each nucleotide of the X and Z domains is a nucleotide of Formula (I). In some embodiments, at least one nucleotide of the X domain and at least one nucleotide of the Z domain are each independently selected from the group consisting of a nucleotide of Formula (II), a nucleotide of Formula (IIIa), and a nucleotide of Formula (IIIb). In some embodiments, each of the at least one nucleotide of the X and Z domains are the same nucleotide. In some embodiments, each nucleotide of the Y domain is linked through thiophosphate intersubunit linkages. In some embodiments, the oligonucleotide is single stranded. In some embodiments, the oligonucleotide is an antisense oligonucleotide. In some embodiments, the oligonucleotide is complementary to a sequence of the HBV genome.

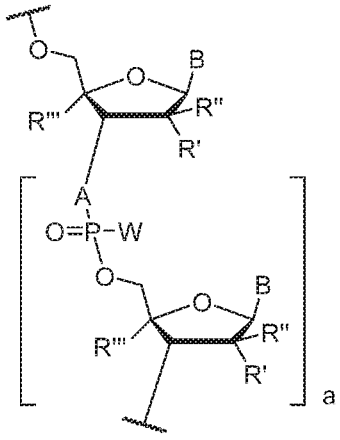
[0008] Another embodiments include a chimeric oligonucleotide represented by Formula (VI):

5' -X—Y—Z- 3' (VI),

wherein X—Y—Z is a chimeric oligonucleotide comprising a sequence of 18 to 22 nucleosides, and is optionally conjugated at the 5' and/or 3' end to a ligand targeting group or a pharmacophore; X is a domain comprising a sequence of modified nucleosides that is 3-10 nucleosides in length; Z is a domain comprising a sequence of modified nucleosides that is 3-10 nucleosides in length; and Y is a domain comprising a sequence of 2 to 14 2'-deoxy-nucleosides linked through thiophosphate intersubunit linkages. In some embodiments, the Y domain is 6 to 10 nucleosides in length. In some embodiments, X and/or Z domains comprise a sequence of modified nucleosides linked through N3'→P5' phosphoramidate or N3'→P5'

thiophosphoramidate intersubunit linkages. In some embodiments, the Y domain comprises at least one phosphodiester intersubunit linkage. In some embodiments, the Y domain consists of 2'-deoxy-nucleosides linked through thiophosphate intersubunit linkages, and optionally one or two phosphodiester intersubunit linkage. In some embodiments, the X domain comprises modified nucleosides where the modification is independently selected from the group consisting of 2'-F, 2'-F-N3'→P5', 2'-OMe, 2'-OMe-N3'→P5', 2'-O-methoxyethoxy, 2'-O-methoxyethoxy-N3'→P5', conformationally restricted nucleosides, 2'-OH-N3′→P5′ thiophosphoramidate and 2'-OH-N3′→P5′ phosphoramidate. In some embodiments, the functional domain of Z comprises modified nucleosides where the modification is selected from the group consisting of 2'-F, 2'-F-N3'→P5', 2'-OMe, 2'-OMe-N3'→P5', 2'-O-methoxyethoxy, 2'-O-methoxyethoxy-N3'→P5', conformationally restricted nucleosides, 2'-OH-N3'→P5' thiophosphoramidate and 2'-OH-N3'→P5' phosphoramidate. In some embodiments, the X and/or Z domains comprise one or more 2'-deoxy-nucleosides linked through a N3'→P5' phosphoramidate intersubunit linkage. In some embodiments, the X and Z domains comprise one or more 2'-arabino-F and/or 2'-ribo-F modified nucleoside, wherein each said nucleoside is independently linked through at least one of an N3′→P5′ phosphoramidate or N3′→P5′ thiophosphoramidate intersubunit linkage. In some embodiments, the X and Z domains comprise one or more 2'-OMe modified nucleosides, wherein each said nucleoside is independently linked through at least one of N3′→P5′ phosphoramidate, N3′→P5′ thiophosphoramidate, or thiophosphate intersubunit linkages. In some embodiments, the modified nucleosides in each of the X and Z domains are 2'-OMe modified nucleosides linked through thiophosphate intersubunit linkages, and wherein the modified nucleosides include 5-methylcytosine nucleobases, but optionally not cytosine. In some embodiments, the modified nucleosides include 2,6-diaminopurine nucleobases, but optionally not adenine. In some embodiments, the modified nucleosides include 5-methyluracil nucleobases, but optionally not uracil. In some embodiments, the modified nucleosides include 2,6-diaminopurine nucleobases, but not adenine and 5-methyluracil nucleobases, but optionally not uracil. In some embodiments, the Y domain comprises 6-8 2'-deoxy-nucleosides. In some embodiments, the modified nucleosides in each of the X and Z domains comprise 2'-OMe modified nucleosides and conformationally restricted nucleosides optionally linked through thiophosphate intersubunit linkages, and wherein the 2'-OMe modified nucleosides include 5-methylcytosine nucleobases, but optionally not cytosine. In

some embodiments, the modified nucleosides in each of the X and Z domains comprise 2'-OMe and conformationally restricted nucleosides. In some embodiments, the modified nucleosides in each of the X and Z domains comprise conformationally restricted nucleosides and, wherein at least one modified nucleoside includes a N3'→P5' phosphoramidate or a N3'→P5' thiophosphoramidate intersubunit linkage. In some embodiments, the Y domain comprises 7-8 2'-deoxy-nucleosides. In some embodiments, the 2'-OMe modified nucleosides include 5-methyluracil nucleobases, but optionally not uracil. In some embodiments, the Y domain comprises 9-10 2'-deoxy-nucleosides. In some embodiments, the X and Z domains comprise nucleotides represented by the Formula (Ix):



(Ix),

wherein A is independently in each instance NH or O; B is independently in each instance an unmodified or modified nucleobase; W is independently in each instance OR or SR, where R is H or a positively charged counter ion; R' and R'' are each independently in each instance selected from the group consisting of H, F, Cl, OH, OMe, Me, and O-methoxyethoxy; R''' is H, or R' and R''' together form –O-CH_2– or –O-$(CH_2)_2$–, and a is an integer of 3 to 9, wherein when R', R'' and R''' are each H, then A is NH, and optionally when A is O, then W is SR. In some embodiments, the ligand targeting group or a pharmacophore is selected from the group consisting of Chol., Toco, Palm, GalNAc, MGB-1, MGB-2, Acr-, Pyr-, Steroyl, HEG linker, a C7 amino linker, and combinations thereof. In some embodiments, the X and/or Z domain comprises one or more oligonucleotide where the modification is 2'-O-methoxyethoxy-N3'→P5'. In some embodiments, the X domain comprises one or more oligonucleotide where the modification is 2'-O-methoxyethoxy-N3'→P5'. In some embodiments, the Z domain comprises one or more oligonucleotide where the modification is 2'-O-methoxyethoxy-

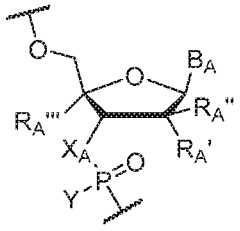
N3'→P5'. In some embodiments, the construct of said oligonucleotide corresponds to a construct of Table B.

[0009] Other embodiments include a chimeric oligonucleotide represented by Formula (VII):

5'-X'—Y'—Z'-3' (VII),

wherein X'—Y'—Z' is a chimeric oligonucleotide comprising a sequence of 16 to 22 nucleosides, and is optionally conjugated at the 5' and/or 3' end; X' is a domain comprising a sequence of modified nucleosides that is 3-10 nucleosides in length; Z' is a domain comprising a sequence of modified nucleosides that is 3-10 nucleosides in length; and Y' is a domain comprising a sequence of 2 to 4 2'-deoxy-nucleosides linked through intersubunit linkages, wherein the X' and/or Z' domains comprise a sequence of modified nucleosides linked through N3′→P5′ phosphoramidate or N3′→P5′ thiophosphoramidate intersubunit linkages. In some embodiments, the Y' domain consists of 2'-deoxy-nucleosides linked through thiophosphate intersubunit linkages, and optionally one phosphodiester intersubunit linkage. In some embodiments, the X' domain is 9 or 10 nucleosides in length. In some embodiments, the X' domain comprises modified nucleosides where the modification is selected from the group consisting of 2'-F, 2'-F-N3'→P5', 2'-OMe, 2'-OMe-N3'→P5', 2'-O-methoxyethoxy, 2'-O-methoxyethoxy-N3'→P5', and conformationally restricted nucleosides. In some embodiments, the Z' domain comprises modified nucleosides where the modification is selected from the group consisting of 2'-F, 2'-F-N3'→P5', 2'-OH, 2'-OMe, 2'-OMe-N3'→P5', 2'-O-methoxyethoxy, 2'-O-methoxyethoxy-N3'→P5', and conformationally restricted nucleosides. In some embodiments, the X' and/or Z' domains comprise one or more 2'-arabino-F and/or 2'-ribo-F modified nucleoside. In some embodiments, the modified nucleosides in the X' and/or Z' domains comprise 2'-OMe and conformationally restricted nucleosides. In some embodiments, the modified nucleosides in the X' and/or Z' domains comprise conformationally restricted nucleosides and a N3'→P5' modification. In some embodiments, the sequence is selected from those in Table C having a 2-4 nucleotide Y domain. Other embodiments include a chimeric oligonucleotide, wherein the sequence of said oligonucleotide corresponds to a sequence listed in Table C.

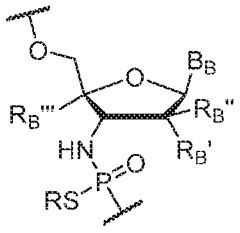
[0010] Other embodiments include an oligonucleotide comprising one or more nucleotides of the following Formula (A):



(VIII),

wherein X_A is NH or O, Y is OR or SR, where R is H or a positively charged counter ion, B_A is independently in each instance a natural or an unmodified nucleobase or a modified nucleobase, R_A' and R_A'' are each independently in each instance selected from H, F, OH, OMe, Me, O-methoxyethoxy, and R_A''' is H or R_A' and R_A''' together form –O-CH_2– or –O-$(CH_2)_2$–. In some embodiments, R_A' and R_A''' are H; and R_A'' is F. In some embodiments, R_A' and R_A'' are H; and R_A''' is F, OH, H or OMe. In some embodiments, X_A is NH; B_A is an unmodified or modified nucleobase; R_A' and R_A''' together form a conformationally restricted nucleoside (e.g., –O-CH_2– or –O-$(CH_2)_2$–); and R_A'' is H. In some embodiments, at least one of R_A' and R_A'' is H. In some embodiments, when B_A is a purine nucleobase at least one of R_A' and R_A'' is OH or F, and/or when B_A is a pyrimidine nucleobase at least one of R_A' and R_A'' is OMe, OH or F. In some embodiments, the modified nucleobase is selected from 5-methylcytosine, 2,6-diaminopurine, 5-methyluracil, and a g-clamp. In some embodiments, the nucleotides of Formula (A) include those in Table G. In some embodiments, the nucleotide of Formula (A) includes a sequence listed in Table H. In some embodiments, the nucleotide of Formula (A) includes a sequence 1, 2, 3, 4, or 5 nucleobases different from a sequence selected from those in Table B.

[0011] Other embodiments include an oligonucleotide comprising ten or more nucleotides of the following Formula (IX):



(IX),

wherein R is H or a positively charged counter ion, B_B is independently in each instance a natural or an unmodified nucleobase or a modified nucleobase, R_B' and R_B'' are each independently in each instance selected from H, F, OMe, Me, O-methoxyethoxy, and R_B''' is H or R_B' and R_B''' together form –O-CH_2– or –O-$(CH_2)_2$–. In some embodiments, R_B' and R_B''' are H; and R_B'' is

F. In some embodiments, R_B' and R_B'' are H; and R_B''' is F, OH, H or OMe. In some embodiments, B_B is an unmodified or modified nucleobase; R_B' and R_B''' together form a conformationally restricted nucleoside (e.g., –O-CH_2– or –O-$(CH_2)_2$–); and R_B'' is H. In some embodiments, at least one of R_B' and R_B'' is H. In some embodiments, when B_B is a purine nucleobase at least one of R_B' and R_B'' is OH or F, and/or when B_B is a pyrimidine nucleobase at least one of R_B' and R_B'' is OMe, OH or F. In some embodiments, the modified nucleobase is selected from 5-methylcytosine, 2,6-diaminopurine, 5-methyluracil, and a g-clamp. In some embodiments, the nucleotides of Formula (B) include those in Table A where X_A is NH. In some embodiments, the nucleotide of Formula (B) includes a sequence listed in Table B. In some embodiments, the nucleotide of Formula (B) includes a sequence 1, 2, 3, 4, or 5 nucleobases different from a sequence selected from those in Table B. In some embodiments, every oligonucleotide is a nucleotide of the Formula (B).

[0012] Other embodiments include a pharmaceutical composition comprising an oligonucleotide of any of the preceding embodiments and a pharmaceutically acceptable excipient. In some embodiments, the composition is suitable for intravenous or subcutaneous delivery. Other embodiments include a method of inhibiting Hepatitis B virus (HBV) gene expression in a cell comprising contacting the cell with an oligonucleotide or composition of any of the preceding embodiments. Other embodiments include a method of inhibiting replication of a Hepatitis B virus (HBV) in a cell comprising contacting the cell with an oligonucleotide or composition of any of the preceding embodiments. Other embodiments include a method of treating a subject having a Hepatitis B virus (HBV) infection, comprising administering to the subject a therapeutically effective amount of an oligonucleotide or composition of any of the preceding embodiments. Other embodiments include a, oligonucleotide of any of the preceding embodiments, wherein said oligonucleotide complexed with an HBV genome sequence has a melting temperature (Tm) of >37 °C. Other embodiments include a method of treating a subject having a Hepatitis B virus (HBV) infection, comprising administering to the subject a therapeutically effective amount of an oligonucleotide or composition of any of the preceding embodiments. Other embodiments include a method of inhibiting expression of a target RNA in a cell comprising contacting the cell with an oligonucleotide or composition comprising said oligonucleotide of any of the preceding embodiments, wherein the chimeric oligonucleotide contains a nucleobase sequence that is complementary or hybridizes to a portion of the target

RNA. Other embodiments include a method of inhibiting replication of a virus in a cell comprising contacting the cell with an oligonucleotide or composition comprising said oligonucleotide of any of the preceding embodiments, comprising said oligonucleotide contains a nucleobase sequence that is complementary or hybridizes to a portion a viral target RNA. Other embodiments include a method of treating a subject having a viral infection, comprising administering to the subject a therapeutically effective amount of an oligonucleotide or composition comprising said oligonucleotide of any of the preceding embodiments, wherein the oligonucleotide contains a nucleobase sequence that is complementary or hybridizes to a portion of viral target RNA. Other embodiments include a method of modulating expression of a target by contacting a target nucleic acid with an antisense compound comprising an oligonucleotide or composition comprising said oligonucleotide of any of the preceding embodiments, wherein the oligonucleotide contains a nucleobase sequence that is complementary or hybridizes to a portion of target nucleic acid.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1A shows HBsAg serum levels. FIG. 1B shows HBeAg serum levels. FIG. 1C shows DNA serum levels.

[0014] FIG. 2A show results HBsAg serum levels for a GalNAc conjugated compound of the present disclosure for IV administration. FIG. 2B shows HBsAg serum levels for a GalNAc conjugated compound of the present disclosure for SC administration.

[0015] FIG. 3 shows HBsAg reduction levels for GalNAc conjugated compounds of the present disclosure.

[0016] FIGs. 4A-4C show *in vivo* HBsAg, HBeAg and Serum HBV DNA data in an AAV-HBV mouse model for compounds of the present disclosure. FIG. 4A shows HBsAg serum levels. FIG. 4B shows HBeAg serum levels. FIG. 4C shows HBV DNA levels.

[0017] FIGs. 5A-5C show *in vivo* HBsAg, HBeAg and serum HBV DNA data in an AAV-HBV mouse model for compounds of the present disclosure. FIG. 5A shows HBsAg serum levels. FIG. 5B shows HBeAg serum levels. FIG. 5C shows HBV DNA levels.

[0018] FIGs. 6A-6C show *in vivo* HBsAg, HBeAg and serum HBV DNA data in an AAV-HBV mouse model for compounds of the present disclosure. FIG. 6A shows HBsAg serum levels. FIG. 6B shows HBeAg serum levels. FIG. 6C shows HBV DNA levels.

[0019] FIG. 7 shows various compounds of the present disclosure and their respective complimentary sites for the HBV (+) strand genome.

[0020] FIG. 8 shows HBsAg level in serum for two oligonucleotides described in Table 29.

[0021] FIG. 9A shows HBsAg level in serum for two oligonucleotides described in Table 30. FIG. 9B shows HBeAg level in serum for two oligonucleotides described in Table 30.

[0022] FIG. 10A shows HBsAg level in serum for two oligonucleotides described in Table 31. FIG. 10B shows HBeAg level in serum for two oligonucleotides described in Table 31.

[0023] FIG. 11A shows HBsAg level in serum for oligonucleotides described in Table 33 as a single dose. FIG. 11B shows HBsAg level in serum for oligonucleotides described in Table 33 for a dosing regimen of 3x3.3 mg/kg on Days 0, 2, 4.

[0024] FIG. 12A shows HBsAg level in serum for oligonucleotides described in Table 37 as a single dose. FIG. 12B shows HBsAg level in serum for oligonucleotides described in Table 38 as a single dose. FIG. 12C shows HBsAg level in serum for oligonucleotides described in Table 40 as a single dose.

DETAILED DESCRIPTION

[0025] The present disclosure is directed to modified nucleotides and oligonucleotides comprising the modified nucleotides and modified linkages between nucleotides. The present disclosure is also directed to constructs of the oligonucleotides, which include domains, regions or portions within the oligonucleotide having common features and additional components conjugated to the oligonucleotide such as targeting moieties. The present disclosure is further directed to methods of using and preparing the oligonucleotides and their constructs.

[0026] As known in the art and as set forth in the present disclosure, a modified nucleotide is any nucleotide that is not a deoxyribonucleotide. For example, the 2' carbon of the deoxyribose may be substituted by a substituent other than the hydroxy (OH); the 3' carbon of the deoxyribose may be substituted by a substituent other than the oxygen atom (O). As known in the art and as set forth in the present disclosure, a modified linkage between two nucleotides is any linkage that

is not a phosphodiester bond between the 3' carbon of the deoxyribose of the first nucleotide and the 5' carbon of the deoxyribose of the second nucleotide.

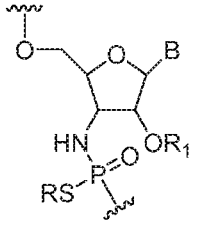
1. 2', 3'-Modified Nucleotides and Related Oligonucleotides

[0027] Compounds of the present disclosure include modified nucleotides with particular 2' and 3' modifications. In embodiments, compounds of the present disclosure include replacement of the hydroxy, or substitution, at the 2' carbon of the deoxyribose sugar. In addition, these compounds of the present disclosure include modifications of the linkage between two nucleosides, which includes replacement of the oxygen atom, or substitution, with a nitrogen atom (N) at the 3' carbon of the deoxyribose sugar. Modifications of the linkage further include replacement of another oxygen atom, or substitution, in the phosphodiester bond.

[0028] These modified nucleotides may be used, e.g., in oligonucleotides such as chimeric oligonucleotides allowing for enzymatic cleavage of the genetic target by RNase H or modified antisense oligonucleotides.

A. 2', 3'-Modified Nucleotides

[0029] Accordingly, compounds of the present disclosure include nucleotides of Formula (I):

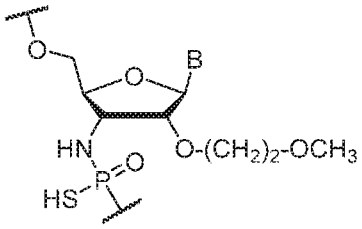


(I),

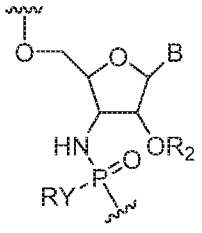
wherein R is H or a positively charged counter ion, B is independently in each instance a natural or an unmodified nucleobase or a modified nucleobase, R_1 is $-(CR'_2)_2OCR'_3$, and R' is independently in each instance H or F.

[0030] In nucleotides of Formula (I), R_1 is $-(CR'_2)_2OCR'_3$. In some embodiments, R' is H in each instance. In other embodiments, at least one R' is F, for example, 1, 2, 3, 4, 5, 6, or 7 R's are F. In some embodiments, CR'_3 contains 1, 2 or 3 F moieties. For example, in embodiments, R_1 is selected from the group consisting of $-CH_2CH_2OCH_3$ (or MOE), $-CF_2CH_2OCH_3$, $-CH_2CF_2OCH_3$, $-CH_2CH_2OCF_3$, $-CF_2CF_2OCH_3$, $-CH_2CF_2OCF_3$, $-CF_2CH_2OCF_3$, $-CF_2CF_2OCF_3$,

–$CHFCH_2OCH_3$, –$CHFCHFOCH_3$, –$CHFCH_2OCFH_2$, –$CHFCH_2OCHF_2$ and –$CH_2CHFOCH_3$. In embodiments, the nucleotide of Formula I is:



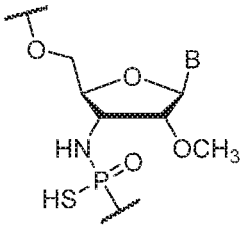
[0031] In embodiments, compounds of the present disclosure include at least one nucleotide of Formula (I) and at least one nucleotide of Formula (II):



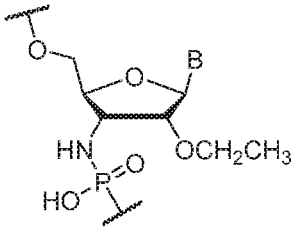
(II),

wherein Y is S or O, R is H or a positively charged counter ion, B is a nucleobase, R_2 is –CR'_3, –$CR'_2OCR'_3$, –$(CR'_2)_3OCR'_3$ or –$(CR'_2)_{1\text{-}2}CR'_3$, or R_2 is –$(CR'_2)_2OCR'_3$ and Y is O and R' is independently in each instance H or F.

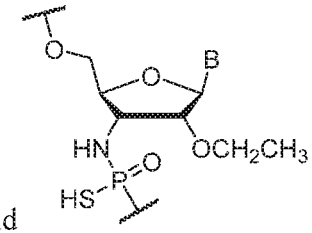
[0032] In the nucleotide of Formula (II), R_2 is –CR'_3, –$(CR'_2)_{1\text{-}3}OCR'_3$, or –$(CR'_2)_{1\text{-}2}CR'_3$. In some embodiments, R_2 is–CR'_3 or –$CR'_2CR'_3$. In some embodiments, R' is H in each instance. In other embodiments, at least one R' is F, for example, 1, 2, 3, 4, or 5 R's are F. In some embodiments, CR'_3 contains 1, 2 or 3 F moieties. For example, in embodiments, R_1 is selected from the group consisting of –CH_3 (or Me), –CFH_2, –CHF_2, CF_3, –CH_2OCH_3, –CFH_2OCH_3, –CHF_2OCH_3, –CF_3OCH_3, –CH_2OCFH_2, –CH_2OCHF_2, –CH_2OCF_3, –CFH_2OCH_3, –CFH_2OCFH_2, –CFH_2OCHF_2, –CFH_2OCF_3, –CHF_2OCH_3, –CHF_2OCFH_2, –CHF_2OCHF_2, –CHF_2OCF_3, –$(CR'_2)_3OCR'_3$, –CH_2CH_3 (or Et), –CFH_2CH_3, –CHF_2CH_3, –CF_3CH_3, –CH_2CFH_2, –CH_2CHF_2, –CH_2CF_3, –CFH_2CH_3, –CFH_2CFH_2, –CFH_2CHF_2, –CFH_2CF_3, –CHF_2CH_3, –CHF_2CFH_2, –CHF_2CHF_2, –CHF_2CF_3, –$CH_2CH_2CH_3$, $CF_2CH_2CH_3$, $CH_2CF_2CH_3$, $CH_2CH_2CF_3$, $CF_2CF_2CH_3$, $CH_2CF_2CF_3$, $CF_2CH_2CF_3$, $CF_2CF_2CF_3$, $CHFCH_2CH_3$, $CHFCHFOCH_3$, $CHFCH_2CFH_2$, $CHFCH_2CHF_2$ and CH_2CHFCH_3. In embodiments, R_1 is –CH_3 (or Me) or –CH_2CH_3 (or Et). In embodiments, the nucleotides of Formula II are selected from the group consisting of



,



and



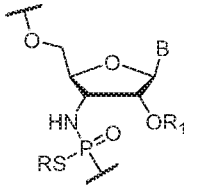
.

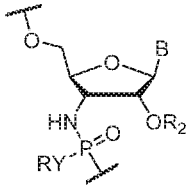
[0033] In compounds of Formulae (I) or (II), Y may be O or S. In some embodiments, Y is S in at least one instance (e.g., 1, 2, 3, 4, 5, 6,7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 etc.). In other embodiments, Y is S in at least one instance and O in at least another instance. In other embodiments, Y is S in each instance. In some embodiments, Y is O in at least one instance (e.g., 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 etc.).

[0034] The disclosed oligonucleotides comprise at least one nucleotide of Formula (I). In embodiments, the disclosed oligonucleotides comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 nucleotides of Formula (I). In embodiments, the disclosed oligonucleotides comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 nucleotides of Formula (II). In some embodiments, the oligonucleotide comprises from 2 to 40 nucleotides, for example, 8 to 26 nucleotides or integers there between.

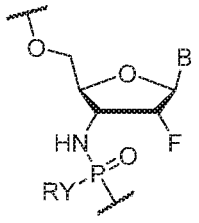
[0035] In embodiments where more than one nucleotide of Formula (I) are included, the nucleotide may be the same or different. In some embodiments one or more nucleotides of Formula (II) are included, and may be the same or different. For example, in some embodiments, the oligonucleotide comprises at least one nucleotide of Formula (I) and at least one nucleotide of Formula (II). In some embodiments, the oligonucleotide comprises at least one nucleotide of Formula (I), wherein at least one R_1 is MOE and at least one nucleotide of Formula (II), wherein R_2 is Me or Et. In some embodiments, the oligonucleotide comprises at least 2 alternating nucleotides of Formula (I) and Formula (II). For example, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 nucleotides with alternating 2' modification (e.g., Me-MOE-Me-MOE… or Et-MOE-Et-MOE-Et-MOE…).

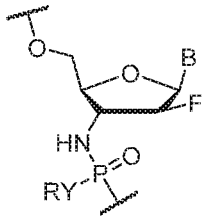
[0036] In some embodiments, the nucleotide of Formula (I) and/or Formula (II) is represented by the following:


(I')


(II'),

[0037] In some embodiments, the oligonucleotide comprising the nucleotide of Formula (I) further comprises a 2'-fluoronucleotide of the Formula (IIIa) and/or (IIIb):

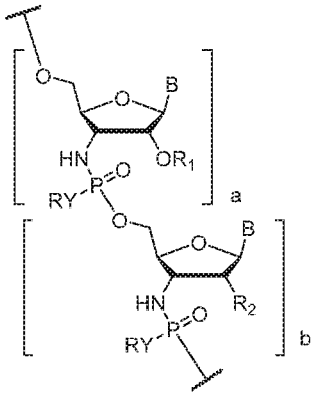

(IIIa)


(IIIb),

wherein Y is S or O, R is H or a positively charged counter ion, and B is a nucleobase.

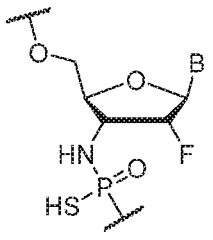
[0038] In some embodiments, the oligonucleotide comprises at least 4 alternating nucleotides of Formulae (I) and (IIIa). For example, the oligonucleotide comprises 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 alternating nucleotides.

[0039] Certain embodiments include an oligonucleotide comprising 4-40 nucleotides, and comprising Formula (IV):


(IV),

wherein Y is S or O, R is H or a positively charged counter ion, B is a nucleobase, R_1 is $-(CR'_2)_2OCR'_3$, R_2 is selected from $-OCR'_3$, $-OCR'_2OCR'_3$, $-O(CR'_2)_3OCR'_3$ or $-O(CR'_2)_{1-2}CR'_3$ and F, R' is independently in each instance H or F, and a is an integer of 1-10 and b is an integer from 1-10, where the to 20, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20.

[0040] Compounds of the present disclosure include compounds comprising the following Formula (III'):



(III'),

wherein Y is S or O, R is H or a positively charged counter ion, and B is independently in each instance a natural or an unmodified nucleobase or a modified nucleobase; and optionally comprising one or more of formula (I), (II) , and/or (IV).

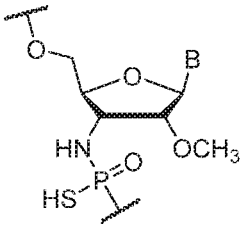
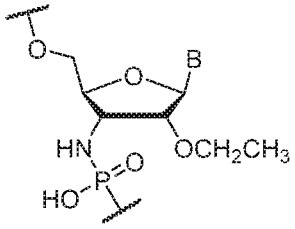
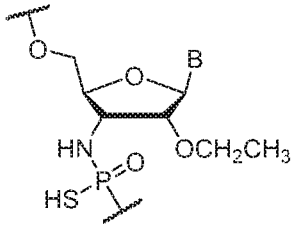
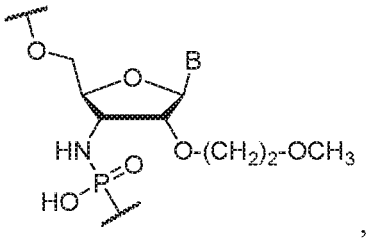
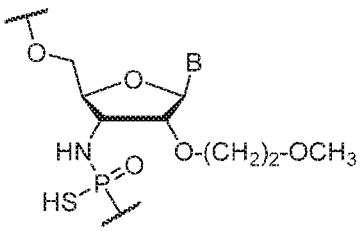
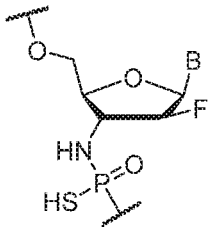
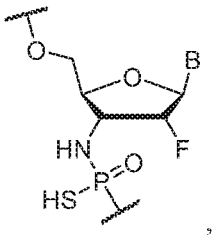
[0041] The nucleobases, B, of the nucleotides of Formulae (I), (II), (IIIa), (IIIb), (IV) and (V) may each independently be a natural or an unmodified nucleobase or a modified nucleobase. In some embodiments, the modified nucleotides include 2,6-diaminopurine nucleobases, but optionally not adenine. In some embodiments, the modified nucleotides include 5-methyluracil nucleobases, but optionally not uracil. In some embodiments, the modified nucleotides include 2,6-diaminopurine nucleobases, but not adenine and 5-methyluracil nucleobases, but optionally not uracil.

[0042] Y in each nucleotide of Formulae (II), (IIIa), (IIIb), (IV) and (V) may be independently O or S. In some embodiments, Y is S in at least one instance (e.g., 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 etc.). In other embodiments, Y is S in at least one instance and O in at least another instance. In other embodiments, Y is S in each instance. In some embodiments, Y is O in at least one instance (e.g., 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 etc.).

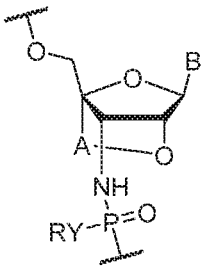
[0043] In embodiments where more than one nucleotide of each of Formulae (I), (II), (IIIa), (IIIb), (IV) and (V) are included, the more than one nucleotides such Formulae may be the same or different. For example, in some embodiments, the nucleotide comprises at least one nucleotide of Formula (II), (III), (IV), (V) and/or (V') in addition to at least one nucleotide of Formula (I). In some embodiments, the nucleotide comprises at least 2 alternating nucleotides of Formula (I) and/or Formula (II) and/or (III) and/or (IV), (V) and/or (V'). For example, disclosed

oligonucleotides may include 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 nucleotides with alternating 2' modifications.

[0044] In embodiments, the nucleotides of the oligonucleotide are selected from the group consisting of:


,

,

,

,

,

and

, where B can be any natural or modified base.

[0045] Compounds of the present disclosure include compounds comprising the following Formula (V'):


(V'),

wherein Y is S or O, R is H or a positively charged counter ion, B is independently in each instance a natural or an unmodified nucleobase or a modified nucleobase, A is $-(CR''R'')_{1-2}-$ and R'' is independently in each instance H, F or Me, and optionally comprising one or more of Formulae (I), (II), (III), (IV) or (V).

[0046] In the compound comprising formula (V'), A is –(CR''R'')$_{1-2}$–. In some embodiments, A is –(CR''R'')– in other embodiments, A is –(CR''R'')$_2$–. R'' is independently in each instance H or Me. In some embodiments, one R'' is Me and remaining are H. In other embodiments, all R'' are H.

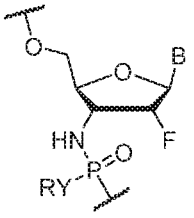
[0047] In some embodiments, when A is CH_2, then Y is S. In other embodiments, when A is CH_2CH_2, then Y is O or S. In some embodiments, A is $CH_2CH(Me)$ or CH(Me) and Y is O or S.

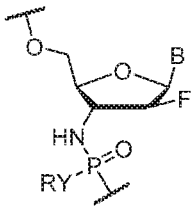
[0048] In the compound comprising formula (V'), Y is O or S. In some embodiments, Y is S in at least one instance (e.g., 1, 2, 3, 4, 5, 6,7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 etc.). In other embodiments, Y is S in at least one instance and O in at least another instance. In other embodiments, Y is S in each instance. In some embodiments, Y is O in at least one instance (e.g., 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 etc.).

[0049] The compound of Formula (V') (and optionally Formulae (I), (II), (III), (IV), (V) and/or (V') may be part of an oligonucleotide. In some embodiments, the compound comprising Formula (IV) (and optionally Formulae (I), (II), (III), (IV) , (V) and/or (V')) is an oligonucleotide comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 nucleotides of Formula (V') (and Formulae (I), (II), (III), (IV), (V) and/or (V')). In some embodiments, the oligonucleotide comprises from 2 to 40 nucleotides, for example, 8 to 26 nucleotides or integers there between.

[0050] In embodiments where more than one nucleotides of Formula (V') are included, the more than one nucleotides of Formula (V') may be the same or different. In some embodiments one or more nucleotides of Formulae (I), (II), (III), (IV), (V) and/or (V') are included, and may be the same or different. For example, in some embodiments, the nucleotide comprises at least one nucleotide of Formula (V') and at least one nucleotide of Formulae (I), (II), (III), (IV) , (V) and/or (V'). In some embodiments, the nucleotide comprises at least 2 alternating nucleotides of Formula (V') and Formula (I) and/or (II). For example, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 nucleotides with alternating 2' modification.

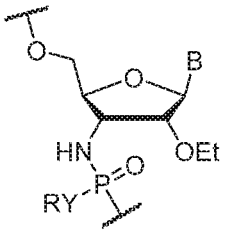
[0051] In some embodiments, the nucleotide comprising the nucleotide of Formula (V') (and optionally Formulae (I), (II), (III), (IV), (V) and/or (V')) further comprises a 2-fluoronucleotide of the following structures:



or


, where Y, R and B are the same as for Formula (I). In some embodiments, the nucleotide comprises at least 4 alternating nucleotides of Formula (V') and 2-fluoronucleotides.

[0052] Compounds of the present disclosure include compounds comprising the following Formula (V):



,

wherein Y is S or O, R is H or a positively charged counter ion, and B is independently in each instance a natural or an unmodified nucleobase or a modified nucleobase; and optionally comprising one or more of formula (I), (II), (III), (IV) and/or (V').

B. Chimeric Oligonucleotides

[0053] The present disclosure is directed to constructs of oligonucleotides, which include domains, regions or portions within the oligonucleotide having common features. Oligonucleotides having these domains are referred to herein as chimeric oligonucleotides. In some embodiments, chimeric oligonucleotides are represented by Formula (VI):

5'-X—Y—Z-3' (VI),

wherein the chimeric oligonucleotide comprises a sequence of 14 to 22 nucleosides, wherein X is a domain comprising a sequence of modified nucleotides that is 3-10 nucleotides in length; Z is a domain comprising a sequence of modified nucleotides that is 3-10 nucleosides in length; and Y is a domain comprising a sequence of 2-10 2'-deoxy- nucleotides, or unmodified nucleotides. Each of the nucleosides in each of the domains is linked through intersubunit linkages.

[0054] In some embodiments, chimeric oligonucleotides are represented by Formula (VI'):

5'-X—Y—Z-3' (VI'),

wherein the chimeric oligonucleotide comprises a sequence of 14 to 22 nucleosides, wherein X is a domain comprising a sequence of modified nucleotides that is 2-10 nucleotides in length; Z is a domain comprising a sequence of modified nucleotides that is 2-10 nucleosides in length; and Y is a domain comprising a sequence of 6-14 2'-deoxy- nucleotides, or unmodified nucleotides. Each of the nucleosides in each of the domains is linked through intersubunit linkages.

[0055] Nucleotides of formula (I), (II), (IIIa), (IIIb), (IV), (V) and/or (V') may be present in the X and/or Z domain. Chimeric oligonucleotide may be conjugated at the 5' and/or 3' end to a ligand-targeting group or a pharmacophore.

[0056] In some embodiments, the Y domain contains 2'deoxy-nucleosides linked by thiophosphate intersubunit linkages. In embodiments, the Y domain contains 2'deoxy-nucleosides linked by at least one phosphodiester intersubunit linkage. In embodiments, the Y domain contains 2'deoxy-nucleosides linked by two phosphodiester intersubunit linkages. In embodiments, the Y domain contains 2'deoxy-nucleosides linked by thiophosphate intersubunit linkages and one or two phosphodiester intersubunit linkages. In some embodiments, the Y domain is 6 to 10 nucleotides in length.

[0057] In some embodiments, the X domain comprises nucleotides of formulae (I), (II), (IIIa), (IIIb), (IV), (V) and/or (V'). In some embodiments, the X domain comprises modified nucleotides where the modification is independently selected from 2'-OMe, 2' -OEt, 2'-O-methoxyethoxy, and conformationally restricted nucleotides. In some embodiments, the X domain is 9 or 10 nucleotides in length.

[0058] In some embodiments, the Z domain comprises nucleotides of formulae (I), (II), (IIIa), (IIIb), (IV), (V) and/or (V'). In some embodiments, the Z domain comprises 2' modified nucleotides where the modification is 2'-OMe, 2' -OEt or 2'-MOE. In some embodiments, the Z domain is 9 or 10 nucleotides in length.

[0059] In embodiments, the chimeric oligonucleotide comprises a sequence of 14 to 22 nucleotides. For example, the oligonucleotide may include 14, 15, 16, 17, 18, 19, 20, 21 or 22 nucleotides.

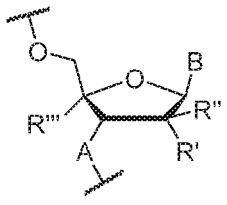
[0060] In embodiments, X is a domain consisting of a sequence containing one or more modified nucleotides that is 3-10 nucleotides in length; Z is a domain consisting of a sequence containing one or more modified nucleotides that is 3-10 nucleotides in length; and Y is a domain consisting of a sequence of 2 to 10 2'-deoxy-nucleosides linked through thiophosphate intersubunit linkages and optionally one or two phosphodiester intersubunit linkages. In some embodiments, X is 5-9, Y is 6-10 and Z is 5-9. In some embodiments, the number of nucleotides in each of X, Y and Z, respectively is: 6/6/6, 6/6/7, 6/6/8, 6/7/6, 6/7/7, 6/7/8, 6/8/6, 6/8/7, 6/8/8, 3/10/3, 4/10/4, 5/10/5, 5/10/6, 2/12/2, 3/12/3, 2/14/2, 5/9/5, 5/9/6, 5/8/5, 5/8/6, 5/8/7, 7/5/7, 7/5/8, 7/5/9,7/6/6, 7/6/7, 7/6/8, 7/6/9, 7/7/6, 7/7/7, 7/7/8, 7/7/9, 7/5/7, 7/5/8, 7/5/9, 7/4/7, 7/4/8, 7/4/9, 8/4/7, 8/4/8, 8/4/9, 7/3/7, 7/3/8, 7/3/9, 8/3/7, 8/3/8, 8/3/9, 8/3/10, 9/3/7, 9/3/8, 9/3/9, 9/3/10, 8/2/7, 8/2/8, 8/2/9, 8/2/10 ,9/2/7, 9/2/8, 9/2/9, 9/2/10, 10/2/8, 10/2/9, 10/2/10. The X domain and the Z domain each, respectively, comprise a sequence of modified nucleotides, where the domain is 4-10 nucleotides in length. For example, the X domain and/or Z domain may comprise a sequence of 4, 5, 6, 7, 8, 9, or 10 nucleotides. One or more of these nucleotides is modified (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or10). For example, in some embodiments, all the nucleotides in each of the X domain and/or Z domain are modified.

[0061] The nucleotides of the X and Z domains may be modified according to Formulae (I), (II), (IIIa), (IIIb), (IV), , (V) and/or (V') with respect to one or more of their nucleobases, the 2' and/or 3' positions on the ribose sugar and their intersubunit linkages. Embodiments include wherein the 2' position is modified with an F (ribo or arabino) and the 3' position is O or NH. Embodiments also include wherein the 2' position is modified with an OMe and the 3' position is O or NH. Embodiments include wherein the 2' position is modified with an F (ribo or arabino) as well as Me or OMe, and the 3' position is O or NH. Embodiments include wherein the 2' position is modified with an F (ribo or arabino) and the 3' position is O or NH. Embodiments include wherein the 2' position is modified with an O-methoxyethoxy and the 3' position is O or NH. Embodiments also include wherein the 2' position is modified with an F (ribo or arabino) and the 3' position is O or NH. Embodiments include wherein the 2' and 4' positions are modified bridging group (as described elsewhere herein) to form a conformationally restricted nucleotide and the 3' position is O or NH. Each of these embodiments may include thiophosphate (or thiophosphoramidate depending on the 3' substitution) and phosphoramidate intersubunit linkages.

[0062] Embodiments also include where the 2' position is H, and the 3' position is NH. Each of these embodiments may include thiophosphoramidate and/or phosphoramidate intersubunit linkages.

[0063] In some embodiments, the modified nucleotides of the X domain and the Z domain each, respectively, include a modification independently selected from at least one of 2'-F, 2'-F-N3'→P5', 2'-OMe, 2'-OMe-N3'→P5', 2'-O-methoxyethoxy, 2'-O-methoxyethoxy-N3'→P5', conformationally restricted nucleotides.

[0064] In some embodiments, the modified nucleotide contains a nucleoside represented by the following Formula (A):



(A),

wherein A is independently in each instance NH or O, B is independently in each instance a natural or an unmodified nucleobase or a modified nucleobase, and R' and R'' are each independently in each instance selected from H, F, OH, OMe, OEt, O-methoxyethoxy, and R''' is H, or R' and R''' together form a 2-4 atom bridge to form a conformationally restricted nucleoside (e.g., –O-CH_2–, –O-CH(Me)–, or –O-$(CH_2)_{2}$–).

[0065] In some embodiments, R' is selected from F, OH, -OMe, -OEt, O-methoxyethoxy; R'' is H and F; and R''' is H, Me or -OMe. In other embodiments, R'' and R''' are H; and R' is selected from F, OMe, OEt and O-methoxyethoxy. In some embodiments, A is NH in each instance.

[0066] Some embodiments include one or more modified nucleosides represented by Formula (A), wherein A is NH; B is a G-clamp; R' is F or OMe and R'' is H; or R' is H and R'' is H or F; and R''' is H.

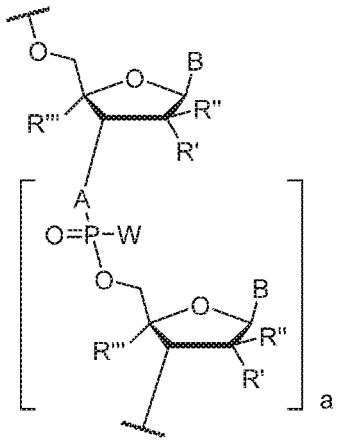
[0067] Some embodiments include one or more modified nucleosides represented by Formula (A), wherein A is NH; B is an unmodified or modified nucleobase; R' and R''' together form a conformationally restricted nucleoside (e.g., –O-CH_2–, –O-CH(Me)–, or –O-$(CH_2)_{2}$–); and R''

is H. In some embodiments, B is an unmodified or a modified nucleobase selected from the group consisting of 5-methylcytosine, 2,6-diaminopurine, and 5-methyluracil.

[0068] Some embodiments include one or more modified nucleosides represented by Formula (A), wherein A is NH; B is an unmodified or modified nucleobase; R' is F or OMe, R'' is H and R''' is H.

[0069] Some embodiments include one or more modified nucleosides represented by Formula (A), wherein A is NH; B is an unmodified or modified nucleobase; R' is H, R'' is F and R''' is H.

[0070] In some embodiments, the X and Z domains are represented by the Formula (Ix):

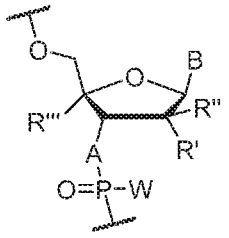


(Ix),

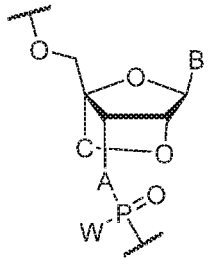
wherein W is independently in each instance OR or SR, where R is H or a positively charged counter ion; R', R'', R''', A and B are as described for Formula (A). In other embodiments, A is O and R', R" are independently H or OEt, where at least one of R', R" is OEt.

[0071] For example, the nucleotides of X and/or Z may include one or more of the nucleotides in Table A in addition to at least one nucleotide in each of the X and Z domains where A is NH, W is S, and R' is MOE.

Table A



Nucleotide No.	R'	R''	R'''	A	W
1	F	H	H	NH	S
2	F	H	H	NH	O
3	F	H	H	O	S
4	F	H	H	O	O
5	H	F	H	NH	S
6	H	F	H	NH	O
7	H	F	H	O	S
8	H	F	H	O	O
9	OMe	H	H	NH	S
10	OMe	H	H	NH	O
11	OMe	H	H	O	S
12	OMe	H	H	O	O
13	H	F	H	NH	S
14	H	F	H	NH	O
15	H	F	H	O	S
16	H	F	H	O	O
17	O-methoxyethoxy	H	H	NH	S
18	O-methoxyethoxy	H	H	NH	O
19	O-methoxyethoxy	H	H	O	S
20	O-methoxyethoxy	H	H	O	O
21	H	H	H	NH	S
22	H	H	H	NH	O
23	OH	H	H	NH	S
24	OH	H	H	NH	O
25	OH	H	H	O	S
26	H	OH	H	NH	O
27	H	OH	H	NH	S
28	H	OEt	H	NH	O
29	H	OEt	H	NH	S
30	H	OEt	H	O	O
31	H	OEt	H	O	S
32	OEt	H	H	NH	O
33	OEt	H	H	NH	S
34	OEt	H	H	O	O
35	OEt	H	H	O	S



Nucleotide No.	C	A	W
36	-O-CH_2-	NH	S
37	-O-CH_2-	NH	O
38	-O-CH_2-	O	S
39	-O-CH_2-	O	O
40	-O-$(CH_2)_2$-	NH	S
41	-O-$(CH_2)_2$-	NH	O
42	-O-$(CH_2)_2$-	O	S
43	-O-$(CH_2)_2$-	O	O
44	-O-CH(Me)-	NH	S
45	-O-CH(Me)-	NH	O
46	-O-CH(Me)-	O	S
47	-O-CH(Me)-	O	O

[0072] In some embodiments, the X domain and Z domain each independently comprise two, three or more different nucleotides 1-47.

[0073] The nucleosides of the X domain are linked through intersubunit linkages, for example, N3′→P5′ phosphoramidate, N3′→P5′ thiophosphoramidate, thiophosphate, phosphodiester intersubunit linkages or combinations thereof. In some embodiments, the X domain is linked through intersubunit linkages selected from N3′→P5′ phosphoramidate, N3′→P5′ thiophosphoramidate, and combinations thereof.

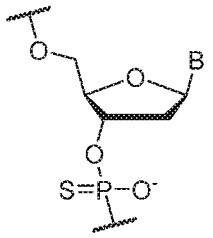
[0074] The X domain of the chimeric oligonucleotide may include a certain arrangement of modified nucleotides. For example, in some embodiments, the X domain comprises one or more conformationally restricted nucleotides. Conformationally restricted nucleotides can include BNA, such as, LNA and ENA. (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 conformationally restricted nucleotides). In some embodiments, the X domain comprises one or more 2'-F and/or 2'-OMe modified nucleotides. In some embodiments, the X domain comprises alternating conformationally restricted nucleotides, e.g., every other nucleotide is a conformationally restricted nucleotide. In some embodiments, the X domain comprises one or more 2'-F and/or 2'-

OMe modified nucleotide (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 2'-F and/or 2'-OMe modified nucleotides). In some embodiments, the X domain comprises alternating 2'-F and 2'-OMe modified nucleotides. In embodiments, the X domain comprises 2'-F or 2'-OMe and conformationally restricted nucleotides, for example, in an alternating sequence.

[0075] The Y domain comprises a sequence of 2 to 14 2'-deoxynucleotides. For example, the Y domain may comprise a sequence of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 2'-deoxynucleotides. One or more of the 2'-deoxynucleosides may be linked through thiophosphate intersubunit linkages (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 thiophosphate intersubunit linkages). In some embodiments, each of the 2'-deoxynucleosides is linked through a thiophosphate intersubunit linkage. In some embodiments, the Y domain comprises at least one phosphodiester intersubunit linkage (e.g., 1, 2 or 3 phosphodiester intersubunit linkages). In other embodiments, the Y domain consists of 2'-deoxy-nucleosides linked through thiophosphate intersubunit linkages, and optionally one or two phosphodiester intersubunit linkages.

[0076] In embodiments, the Y domain comprises nucleotides that induce RNase H cleavage.

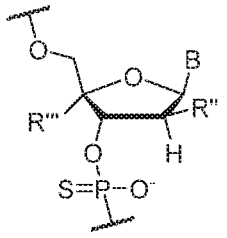
[0077] In some embodiments, the 2'-deoxynucleoside linked through a thiophosphate intersubunit linkage may be represented by the following Formula (B):



(B)

where B is independently in each instance an unmodified or modified nucleobase. In some embodiments, B is an unmodified or a modified nucleobase selected from the group consisting of 5-methylcytosine, 2,6-diaminopurine, and 5-methyluracil.

[0078] In other embodiments, the 2'-deoxynucleoside linked through a thiophosphate intersubunit linkage comprises a modified 2'-deoxynucleoside, which may be modified in the same manner as in the X and Z domain. For example, the modified 2'-deoxynucleoside linked through a thiophosphate intersubunit linkage may be represented by the following Formula (C):



(C)

wherein B is independently in each instance an unmodified or modified nucleobase, and R'' and R''' are each independently in each instance selected from H, F, Cl, OH, OMe, Me, O-methoxyethoxy, or R' and R''' together form a 2-4 atom bridge to form a conformationally restricted nucleoside. In some embodiments, B is an unmodified or a modified nucleobase selected from the group consisting of 5-methylcytosine, 2,6-diaminopurine, and 5-methyluracil.

[0079] The Z domain comprises a sequence of modified nucleotides, where the Z domain is 4-10 nucleotides in length. For example, the Z domain may comprise a sequence of 4, 5, 6, 7, 8, 9, or 10 nucleotides. One or more of these nucleotides is modified (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22). For example, in some embodiments, all the nucleotides in the Z domain are modified.

[0080] The modified nucleotides of the Z domain include, for example, a modification independently selected from at least one of 2'-F, 2'-F-N3'→P5', 2'-OMe, 2'-OMe-N3'→P5', 2'-OEt-N3'→P5', 2'-O-methoxyethoxy, 2'-O-methoxyethoxy-N3'→P5', conformationally restricted nucleotides, 2'-OH-N3'→P5' thiophosphoramidate and 2'-OH-N3'→P5' phosphoramidate.

[0081] In some embodiments, the modified nucleotide may include a nucleoside represented by Formula (A).

[0082] The nucleotides of the Z domain are linked through intersubunit linkages, for example, N3'→P5' phosphoramidate, N3'→P5' thiophosphoramidate, thiophosphate or phosphodiester intersubunit linkages. In some embodiments, the Z domain is linked through N3'→P5' phosphoramidate, N3'→P5' thiophosphoramidate, intersubunit linkages, and combinations thereof.

[0083] The Z domain of the chimeric oligonucleotide may include a certain arrangement of modified nucleotides. For example, in some embodiments, the Z domain comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, or more) conformationally restricted nucleotides (e.g., BNA,

such as, LNA, ENA, each of which may be optionally substituted). In some embodiments, the Z domain comprises alternating conformationally restricted nucleotides, e.g., every other nucleotide is a conformationally restricted nucleotide (e.g., BNA, such as, LNA, ENA, each of which may be optionally substituted). In some embodiments, the Z domain comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, or more) 2'-F and/or 2'-OMe modified nucleotide. For example, some embodiments include where the Z domain comprises alternating 2'-F and 2'-OMe modified nucleotides, or the Z domain comprises alternating 2'-F or 2'-OMe and conformationally restricted nucleotides.

[0084] In some embodiments, the modified nucleotides of Formula (VI) or (VI') include 5-methylcytosine nucleobases, but not cytosine. In some embodiments, the modified nucleotides of Formula (VI) or (VI') include 2,6-diaminopurine nucleobases, but not adenine. In some embodiments, the modified nucleotides of Formula (VI) or (VI') include 5-methyluracil nucleobases, but not uracil. In some embodiments, the modified nucleotides of Formula (VI) or (VI') include 2'-OMe and conformationally restricted nucleotides, and are linked through thiophosphate intersubunit linkages, and the modified nucleotides include 5-methylcytosine nucleobases, but not cytosine. In some embodiments, the modified nucleotides of Formula (VI) or (VI') include the 2'-OMe modified nucleotides with 5-methyluracil nucleobases, but not uracil.

[0085] In certain embodiments, the chimeric oligonucleotide represented by Formula (VI) or (VI') is arranged according to at least one of the constructs of Table B where at least one intersubunit linkage in the X and Z domains is an NPS linkage.

Table B

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	2	ps	A, G, C, T, U	11	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	2	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP,
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	2	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clam, DAP	2	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp	2	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	3	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	3	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	3	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	3	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	3	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	4	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	4	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	4	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	4	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	4	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	5	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	5	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC,	5	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC,

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
		5meU, G clamp, DAP						5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	5	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	5	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	6	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC,	7	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC,

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
		5meU, G clamp, DAP						5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	7	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC,	8	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC,

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
		5meU, G clamp, DAP						5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	8	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	6	np. nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	5	np. nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	8	np. nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	7	np, nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	6	np, nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	7	np, nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	6	np. nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	7	np. nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	9	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np, nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	7	np, nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np, nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	5	np. nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np. nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	4	np. nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	9	np, nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	5	np, nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	5	np, nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	10	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	11	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	12	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	13	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	2	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	3	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

X Domain			Y Domain			Z Domain		
Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions	Number of Nucs	Intersubunit Linkages	Nucleobase	Number of Nucs	Intersubunit Linkages	Nucleobase Substitutions
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	10	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	4	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	5	np, nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps. ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	6	np, nps, ps. PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	9	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	5	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	7	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP
6	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP	14	ps	A, G, C, T, U	8	np, nps, ps, PO	A, G, C, T, U, DAP, 5meC, 5meU, G clamp, DAP

[0086] In Table B, the nucleotides in each of the X and Z domains can be one or more of the numbered nucleotides in Table A. In some embodiments, the chimeric oligonucleotides of Table B include at least 1, 2, 3, 4, 5, 6, 7, 8 or more of the modified nucleotides in Table A. In some

embodiments, all of the nucleotides of X and/or Z are modified nucleotides. In some embodiments, the nucleotides in Table B are selected from certain modified nucleotides listed in Table A such as nucleotide numbers 1-4 or 5-8 or 9-12 or 13-16 or 17-20 or 21-24 or 25-28 or 29-30 or 31-32 or 33. In some embodiments the nucleotides in Table B are selected from certain modified nucleotides listed in Table A such as nucleotide numbers 9-12 and 21-28, or 9-12 and 21-24, or 1-4 and 21-28, or 1-4 and 21-24, or 5-8 and 21-28, or 5-8 and 21-24. In some embodiments, the nucleotides in Table B are selected from one or two or three modified nucleotides listed in Table A such as nucleotide numbers 29-31 or 31-32 or 33. In some embodiments, the nucleotides in Table B are selected from certain modified nucleotides listed in Table A such as nucleotide numbers 29 or 31 or 33. The nucleotides in the Y domain of Table B can include nucleotides of Formula B.

[0087] In some embodiments, the oligonucleotide of Table B is conjugated at the 5' and/or 3' end to a ligand-targeting group or a pharmacophore.

[0088] In some embodiments, the nucleotide compounds of the present disclosure include one of the following sequence: 5'-GCAGAGGTGAAGCGAAGUGC-3', or other sequences in Table H (below).

[0089] In some embodiments, the oligonucleotide comprises a sequence in Table C. In table C, X is independently in each instance a natural or an unmodified nucleobase or a modified nucleobase. In some embodiments, each X is independently selected from A, C, G, U, T, 2,6-diaminopurine, a 5-Me pyrimidine (e.g., 5-methylcytosine, 5-methyluracil), and a g-clamp.

Table C

Modified Sequence (5'-3')
5'-mXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmXpsmXpsmX-3'
5'-mXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmXpsmXpsmXps-Chol-3'
5'-mXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmXpsmXpsmX-GalNAc-3'
5'-mXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmXpsmX-3'
5'-mXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmXpsmX-Chol-3'
5'-mXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmXpsmX-GalNAc-3'

Modified Sequence (5'-3')
5'-mXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmXps mX
5'-mXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmXps mX-Chol-3'
5'-mXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmXps mX-GalNAc-3'
5'-XnpsXnpsXnpsXnpsXnpsXpsXpsXpsXpsXpsXpsXpsXnpsXnpsXnpsXnps-3-NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXpsXpsXpsXpsXpsXpsXnpsXnpsXnpsXnpsXnps-3-NH_2-X-3
5'-XnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXpsXpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnps-3-NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXpsXpsXpsXpsXpsXnpsXnpsXnpsXnps-3-NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXpsXpsXpsXpsXpsXnpsXnpsXnpsXnpsXnps-3-NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnps-3 NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnps-3 NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnps-3 NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnps-3 NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnpsXnps-3 NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnps-3 NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnps-3 NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnps-3 NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnpsXnps-3 NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnpsXnps-3 NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnpsXnps-3 NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXpsXpsXpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnps-3 NH_2-X-3'
5'-XnpsXnpsXnpsXnpsXnpsXnpsXpsXpsXpsXpsXpsXpsXnpsXnpsXnpsXnpsXnpsXnpsXnps-3-NH_2-X-3'
5'-mXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmX-3'
5'-mXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmeXps mX-GalNAc-3'
5'-mXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpoGalNAc-3'
5'-mXpsmXpsmXpsmXmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpo GalNAc-3'
5'-mXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXps mXpsmXpoGalNAc-3'
5'-mXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXps

Modified Sequence (5'-3')
mXpsmXpoGalNAc-3'
5'-fXnpsfXnpsfXnpsfXnpsfXnpsfXnpsXpsXpsXpsXpsXpsXpsfXnpsfXnpsfXnpsfXnpsfXnpsfXnpsfXnps-3-NH_2-fX-3'
5'-fXnpsfXnpsfXnpsfXnpsfXnpsfXnpsXpsXpsXpsXpsXpsXpsXpsfXnpsfXnpsfXnpsfXnpsfXnpsfXnps-3-NH_2-fX-3'
5'-fXnpsfXnpsfXnpsfXnpsfXnpsfXnpsXpsXpsXpsXpsXpsXpsXpsXpsfXnpsfXnpsfXnpsfXnpsfXnps-3-NH_2-fX-3'
5'-XnpXnpXnpXnpXnpXnpXpsXpsXpsXpsXpsXpsXpsXnpXnpXnpXnpXnpXnpXnp-3 NH_2-X-3'
5'-XnpsfXnpsfXnpsXnpsfXnpsXnpsXpsXpsXpsXpsXpsXpsfXnpsXnpsfXnpsfXnpsXnpsfXnpsXnps-3 NH_2-fX-3'
5'-XnpfXnpfXnpXnpfXnpXnpXpsXpsXpsXpsXpsXpsfXnpXnpfXnpfXnpXnpfXnpXnp-3 NH_2-fX-3'
5'-XnpsXfXnpsXfXnpsXnspXfXnpsXnpsXpsXpsXpsXpsXpsXpsXfXnpsXnpsXfXnpsXfXnpsXnpsXfXnpsXnpsXfX-3'
5'-XnpXfXnpXfXnpXnpXfXnpXnpXpsXpsXpsXpsXpsXpsXpsXfXnpXnpXfXnpXfXnpXnpXfXnpXnpXfX-3'
5'-XnpXfXnpXfXnpXnpXfXnpXnpXpsXpsXpsXpsXpsXpsXpsXpsXnpXfXnpXfXnpXnpXfXnpXnpXfX-3'
5-mXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmX-GalNAc-3
5'-mXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmX 3'
5'-mXpsmXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmXps mX 3'
5'-mXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmeXpsmX 3'
5'-mXpsmXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmeXpsm X 3'
5'-mXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmX 3'
5'-mXpsmXpsmXpsmXmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmX 3'
5'-mXpsmXpsmXpsmXmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmX 3'
5'-GalNAc-NHC6-psmXpsm5meXpsm5meXpsmXpsm5meXpsXpsXpsXpsXpsXpsXpsXpsXps5meXpsmXpsmXpsm5meXpsmXpsmX 3'
5'-GalNAc-NHC6-psm5meXpsmXpsmXpsmXpsmXpsXpsXpsXpsXps5meXps5meXpsXps5meXpsXpsXpsmXpsmXpsmXpsmXpsmX 3'
5'-GalNAc-NHC6-psmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXps5meXpsXps5meXps5meXps5meXpsm5meXpsmXpsmXpsmXpsmX 3'
5' GalNAc-NHC6-psmXpsmXpsmXpsmXpsmXpsXpsXps5meXpsXps5meXps5meXps5meXps5meXpsXpsXpsmXpsmXpsmXpsm5meXpsmX 3'
5' GalNAc-NHC6-psmXpsmXpsm5meXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXps5meXpsXpsmXpsmXpsmXpsmXpsmX 3'
mXpsmXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmX
mXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmX
mXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmX

Modified Sequence (5'-3')
mXpsmXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmX/GalNAc/
mXpsmXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmX/GalNAc/
mXpsmXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmX/3CholTEG/
mXpsmXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmX/3CholTEG/
mXpsmXpsmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmXpsmX/3CholTEG/
5'-mXps5mmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXp5mmXpsmXpsmXpsmXpsmXpsmXpsmXpsm5meX-3
5'-mXps5mmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXps5mmXpsmXpsmXpsmXpsmXpsmXpsmXps5mmX-CholesXerol-3'
5'-mXps5mmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXp5mmXpsmXpsmXpsmXpsmXpsmXpsmXpsm5mmX-TEG-CholesXerol-3'
5'-mXps5mmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXps5mmXpsmXpsmXpsmXpsmXpsmXpsmXps5mmX-Tocopherol-3'
5'-mXps5mmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXps5mmXpsmXpsmXpsmXpsmXpsmXpsmXps5mmX-TEG-Tocopherol-3'
5'-mXps5mmXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXps5mmXpsmXpsmXpsmXpsmXpsmXpsmXps5mmX-GalNAc-3'
5'-mXpsm5meXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXps5meXpsmXpsmXpsmXpsmXpsmXpsmXpsm5meX-3'
5'-mXpsm5meXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXps5meXpsmXpsmXpsmXpsmXpsmXpsmXpsm5meX-po-Chol-3'
5'-mXpsm5meXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXps5meXpsmXpsmXpsmXpsmXpsmXpsmXpsm5meX-po-Tocopherol-3'
5'-mXpsm5meXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXps5meXpsmXpsmXpsmXpsmXpsmXpsmXpsm5meX-po-GalNAc-3'
5'-mXpsm5meXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXps5meXpsXpsmXpsmXpsmXpsmXpsmXpsm5meX-3'
5'-mXpsm5meXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXps5meXpsXpsmXpsmXpsmXpsmXpsmXpsm5meX-po-Chol-3'
5'-mXpsm5meXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXps5meXpsXpsmXpsmXpsmXpsmXpsmXpsm5meX-po-Tocopherol-3'
5'-mXpsm5meXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXps5meXpsXpsmXpsmXpsmXpsmXpsmXpsm5meX-po-GalNAc-3'
5-mXps2-4-OXH$_2$-XpsmXps 2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsXpsXpsXpsXpsXpsXpsXpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-Xps mX-3
5-mXps2-4-OXH$_2$-XpsmXps 2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsXpsXpsXpsXpsXpsXpsXpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-Xps mX-Chol-3
5-mXps2-4-OXH$_2$-XpsmXps 2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsXpsXpsXpsXpsXpsXpsXpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-Xps mX-XoXo-3
5-mXps2-4-OXH$_2$-XpsmXps 2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsXpsXpsXpsXpsXpsXpsXpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-Xps mX-GalNAc-3
5-mXps2-4-OXH$_2$-XpsmXps 2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsXpsXpsXpsXpsXpsXpsXpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-Xps mX-3

Modified Sequence (5'-3')
5-mXps2-4-OXH$_2$-XpsmXps 2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsXpsXpsXpsXpsXpsXpsXpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-Xps mX-Chol-3
5-mXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsXpsXpsXpsXpsXpsXpsXpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-Xps mX-XoXo-3
5-mXps2-4-OXH$_2$-XpsmXps 2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsXpsXpsXpsXpsXpsXpsXpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-Xps mX-GalNAc-3
5-mXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsXpsXpsXpsXpsXpsXpsXpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsmXps2-4-OXH$_2$-XpsmX-3
5-dXnpsXnpsXnpsXnpsXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXnpsXnpsXnpsXnpsXn-3
5-dXnpsfXnpsXnpsfXnpsfXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsfXnpsfXnpsfXnpsfXnpsfXn 3'
5-fXnpsXnpsfXnpsfXnpsfXnpsfXnpsXpsXpsXpsXpsXpsXpsXnpsfXnpsfXnpsfXnpsfXnpsfXnpsfXnpsXn-3'
5-fXnpsXnpsfXnpsfXnpsfXnpsfXnpsXpsXpsXpsXpsXpsXpsXpsfXnpsfXnpsfXnpsfXnpsfXnpsXn-3'
5'-dXnpmXnpmXnpmXnpmXnpmXnpXpsXpsXpsXpsXpsXpsmXnpmXnpmXnpmXnpmXnpmXnpmXnpmXnpmXnp-3'
5'-dXnpmXnpmXnpmXnpmXnpmXnpXpsXpsXpsXpsXpsXpsXpsmXnpmXnpmXnpmXnpmXnpmXnpmXnpmXnp-3'
5'-dXnpmXnpmXnpmXnpmXnpmXnpXpsXpsXpsXpsXpsXpsXpsXpsmXnpsmXnpmXnpmXnpmXnpmXnpmXnp-3'
5'-dXnpmXnpmXnpmXnpmXnpXpsXpsXpsXpsXpsXpsXpsXpsmXnpmXnpmXnpmXnpmXnpmXnpmXnp-3'
5'-dXnpmXnpmXnpmXnpmXnpXpsXpsXpsXpsXpsXpsXpsXpsXpsmXnpmXnpmXnpmXnpmXnpmXnpmXnp-3'
mXnpsmoeXnpsmoeXnpsmXnpsmoeXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXnpsmXnpsmoeXnpsmXnpsmoeXnp-C6-NH- GalNAc 6
moeXpsmoeXpsmoeXpsmoeXpsmoeXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXpsmoeXpsmoeXpsmoeXpsmoeX-po-GalNAc2
mXnpsmoeXnpsmoeXnpsmXnpsmoeXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXnpsmXnpsmoeXnpsmXnpsmoeXnpo-C6-NH-GalNAc-6
GalNAc -2-pofXnpsfXnpsfXnpsfXnpsfXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsfXnpsfXnpsfXnpsfXnpsfXn
GalNAc2-moeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXn
moeXpsmoeXpsmoeXpsmoeXpsmoeXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXpsmoeXpsmoeXpsmoeXpsmoeX-GalNAc2
GalNAc2-moeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXn
GalNac-moeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXn
GalNAc2-mXnpsmXnpsmXnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXnpsmoeXnpsmoeXnps

Modified Sequence (5'-3')
moeXnpsmoeXn
GalNac6- NH-C6-moeXpsmoeXpsmoeXpsmoeXpsmoeXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXpsmoeXpsmoeXpsmoeXpsmoeX
GalNAc2-mXnpsmXnpsmXnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXnpsmXnpsmXnpsmXnpsmXn
GalNAc2-etoXnpsetoXnpsetoXnpsetoXnpsetoXnpsXps XpsXpsXpsXpsXpsXpsXpsXpsXpsetoXnpsetoXnpsetoXnpsetoXnpsetoXn
GalNAc2-moeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXnpsmXnpsmXnpsmXnpsmXn
moeXpsmoemXpsmoeXpsmoeXpsmoeXpsXpsXpsXpsXpsXpsXpsXps5mXpsXpsXpsmoeXpsmoeXpsmoeXpsmoemX
moeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXn
mXps5mmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXps5mXpsXpsXpsmXpsmXpsmXpsmXps5mmX
mXnpsmXnpsmXnpsmXnps mXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXnpsmXnpsmXnpsmXnpsmXn
GalNAc2-moeXnpsmoeXnpsmoeXnps moeXnpsmoeXnpsXps XpsXpsXps XpsXpsXps XpsXpsXps moeXnpsmoeXnpsmoeXnps moeXnpsmoeXn
GalNAc6-NH-C6-moeXpsmoeXpsmoeXpsmoeXpsmoeXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXpsmoeXpsmoeXpsmoeXpsmoeX
5'moeXpsmoeXpsmoeXpsmoeXpsmoeXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXpsmoeXpsmoeXpsmoeXpsmoeX-GalNAc2
GalNAc2-mXnpsmXnpsmXnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXnpsmXnpsmXnpsmXnpsmXn'
mXpsmXpsmXpsmXpsmXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXpsmXpsmXpsmXpsmX-GalNAc
GalNAc2-moeXnpsmoeXnpsmoeXnps moeXnpsmoeXnpsXpsXpsXpsXpsXpsXpsXps XpsXpsXpsmoeXnpsmoeXnpsmoeXnps moeXnpsmoeXn
moeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnp-C6-NH-GalNAc6
GalNAc2-moeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXnpsmoeXnpsmoeXnps moeXnpsmoeXn
GalNAc-XnpsXnpsXnpsXnpsXnpsXps XpsXpsXpsXpsXpsXpsXpsXpsXpsXnpsXnpsXnpsXnpsXn
GalNAc-mXnpsmXnpsmXnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXnpsmXnpsmXnpsmXnpsmXn
GalNAc-fXnpsfXnpsfXnpsfXnpsfXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsfXnpsfXnpsfXnpsfXnps-

Modified Sequence (5'-3')
3nh2-fX
GalNAc-afXnpsafXnpsafXnpsafXnpsafXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsafXnpsafXnpsafXnpsafXnpsafXnpsafXn
GalNAc-dXnpsXnpsXnpsXnpsXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXnpsXnpsXnpsXnps-3nh2-X
GalNAc-mXnpsmXnpsmXnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXnpsmXnpsmXnpsmXnpsmXn
GalNAc-fXnpsfXnpsfXnpsfXnpsfXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsfXnpsfXnpsfXnpsfXnps-3nh2-fX
GalNAc-mXnpsmXnpsmXnpsmXnpsmXnpsXpsXpsXpsXpsXps5MeXpsXpsXpsXpsXpsmXnpsmXnpsmXnpsmXnpsmXnpsmXn
GalNAc-moeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsXpsXpsXpsXpsXps5MeXpsXpsXpsXpsXpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXn
moeXpsmoeXpsmoeXpsmoeXpsmoeXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXpsmoeXpsmoeXpsmoeXpsmoeX
moeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXn
fXnpsfXnpsfXnpsfXnpsfXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsfXnpsfXnpsfXnpsfXnpsfX-C6-NH-GalNAc6
fXnpsfXnpsfXnpsfXnpsfXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsfXnpsfXnpsfXnpsfXnpsfXnp-C6-NH-GalNAc6
mXnpsmXnpsmXnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXps mXnpsmXnpsmXnps mXnpsmXnp-C6-NH-GalNAc6
mXnpsmXnpsmXnpsmXnpsmXnpsXpXpsXpsXpsXpsXpsXpsXpsXpsXps mXnpsmXnpsmXnpsmXnpsmX-C6-NH-GalNAc6
moeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsXps XpsXpsXps XpsXpsXps XpsXpsXps moeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnp-C6-NH-GalNAc6
moeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsmoeX-C6-NH-GalNAc6
GalNAc2-moeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsXpsXpsXpsXps XpsXpsXps XpsXpsXpsmoeXnpsmoeXnpsmoeXnpsmoeXnpsmoeXn
GalNAc2-etoXnpsetoXnpsetoXnps etoXnpsetoXnpsXpsXpsXpsXpsXpsXpsXps XpsXpsXps etoXnpsetoXnpsetoXnps etoXnpsetoXn
mXnpsmXnps2-4-OCH_2XnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXps2-4-OCH_2XnpsmXnpsmXnpsmXnps3-NH_2mX
mXnpsmXnps2-4-OCH_2CH_2XnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXps2-4-OCH_2CH_2XnpsmXnpsmXnpsmXnps3-NH_2mX
mXnpsmXnps2-4-OCH_2CH_2XnpsmXnps2-4OCH_2CH_2XnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXps2-4-OCH_2CH_2XnpsmXnpsmXnpsmXnps3-NH_2mX
mXnpsmXnpsmXnpsmXnps2-4-OCH_2CH_2XnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXps2-4-OCH_2CH_2XnpsmXnpsmXnpsmXnps3-NH_2mX
5-mXnpsmCnpsmXnpsmXnpsmXnps2-4-OCH_2CH_2XnpsXpsXpsXpsXpsXpsXpsXpsXps mXnpsmXnpsmXnps2-4-OCH_2CH_2XnpsmXnpsmXnps3-NH_2mX-3

Modified Sequence (5'-3')
mXnpsmXnpsmXnpsmCnpsmXnps2-4-OCH_2CH_2XnpsXpsXpsXpsXpsXpsXpsXps2-4-OCH_2CH_2XnpsmXnpsmXnps2-4-OCH_2 CH_2XnpsmXnpsmXnps3-NH_2mX
mXnpsmXnpsmXnpsmXnpsmXnps2-4-OCH_2CH_2XnpsXpsXpsXpsXpsXpsXpsXpsXps2-4-OCH_2 CH_2XnpsmXnps2-4-OCH_2CH_2XnpsmXnpsmXnpsmXnps3-NH_2mX
2-4OCH_2CH_2XnpsmXnpsmXnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXnpsmXnpsmXnps2-4OCH_2CH_2Xnps3-NH_2mX
2-4 OCH_2CH_2XnpsmXnpsmXnps2-4OCH_2CH_2XnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXnps2-4 OCH_2CH_2XnpsmXnps2-4OCH_2CH_2Xnps3-NH_2mX
2-4OCH_2CH_2XnpsmXnps2-4-OCH_2CH_2XnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXnpsmXnpsmXnpsmXnps3-NH_2mX
2-4OCH_2CH_2XnpsmXnpsmXnpsmXnpsmXnps2-4-OCH_2CH_2XnpsXpsXpsXpsXpsXpsXpsXpsXpsmXnpsmXnpsmXnps2-4-OCH_2CH_2XnpsmXnpsm2-4OCH_2CH_2Xnps3-NH_2mX
2-4 OCH_2CH_2XnpsmXnpsmXnpsmXnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXps mXnpsmXnpsmXnpsmXnpsmXnpsm2-4 OCH_2CH_2Xnps3-NH_2mX
mXnpsmXnpsmXnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXnpsmXnpsmXnpsmXnps3-NH_2mX
mXnps2-4 OCH_2CH_2XnpsmXnps2-4 OCH_2CH_2XnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXnpsmXnps2-4 OCH_2CH_2XnpsmXnps3-NH_2mX
2-4 OCH_2CH_2XnpsmXnps2-4OCH_2CH_2XnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsXpsXpsmXnpsmXnps2-4 OCH_2CH_2XnpsmXnps2-$OCH_2CH_2$3-NH_2X
2-4 OCH_2CH_2XnpsmXnps2-4 OCH_2CH_2XnpsmXnpsmCnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsmXnps2-4 OCH_2CH_2XnpsmXnpsmXnpsmXnps2-4 OCH_2CH_2Xnps3-NH_2mX
mXnps2-4OCH_2CH_2XnpsmXnps2-4 OCH_2CH_2 XnpsmXnpsmXnpsXpsXpsXpsXpsXpsXpsXpsXpsmXnps2-4 OCH_2CH_2XnpsmXnpsmXnps2-4OCH_2CH_2XnpsmXnps3-NH_2mX

[0090] In embodiments, each of the nucleotides of a domain are modified. In embodiments, each of the nucleotides of a domain have the same modifications. In embodiments, each of the nucleotides of the X and Z domains are modified. In embodiments, each of the nucleotides of the X and Z domains have the same modifications. In embodiments, each of the nucleotides of a domain are modified with 2' MOE. In embodiments, each of the nucleotides of the X and Z domains are modified with 2' MOE. In embodiments, each of the nucleotides of a domain are modified with 2' OMe. In embodiments, each of the nucleotides of the X and Z domains are modified with 2' OMe. In embodiments, each of the nucleotides of a domain are modified with 2' OEt. In embodiments, each of the nucleotides of the X and Z domains are modified with 2'

OEt. In embodiments, each of the nucleotides of the X and Z domains are linked by an NPS linkage. In embodiments, the X and Z domains have the same number of nucleotides. In embodiments, the X and Z domains each have 4-8 nucleotides. In embodiments, the X and Z domains each have 5-6 nucleotides. In embodiments, the X and Z domains each have 5 nucleotides. In embodiments, the Y domain has at least twice the number of nucleotides as each of the X and Z domains. In embodiments, the Y domain has 8-12 nucleotides. In embodiments, the Y domain has 10 nucleotides. In embodiments, each of the nucleotides of the Y domain are linked by a PS linkage. In embodiments, at least one nucleobase of the oligonucleotide is modified. In embodiments, at least one nucleobase adjacent to the 3' terminal end of the oligonucleotide is modified. In embodiments, at least one nucleobase in the Z domain of the oligonucleotide is modified. In embodiments, at least one nucleobase in the Y domain of the oligonucleotide is modified.

[0091] In some embodiments, the oligonucleotide represented by Formula (VI) or (VI') is selected from Table D. In other embodiments, the oligonucleotide represented by Formula (VI) or (VI') has a sequence that differs from a chimeric oligonucleotide of Table D by one modified nucleotide. In other embodiments, the oligonucleotide represented by Formula (VI) or (VI') has a sequence that differs from an oligonucleotide of Table D by 1, 2, 3 or 4 nucleotides. Specific embodiments of the chimeric oligonucleotide represented by Formula (VI) or (VI') are listed below in Table D:

Table D

#ID	Modified Sequence (5'-3')
101	5'-mGpsmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmApsmApsmGpsmUpsmGpsmC-3'
102	5'-mGpsmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmApsmApsmGpsmUpsmGpsmCps-Chol-3'
103	5'-mGpsmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmApsmApsmGpsmUpsmGpsmC-GalNAc-3'
104	mGpsmApsmUpsmUpsmApsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmApsmApsmApsmG
105	5'-mGpsmApsmUpsmUpsmApsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmApsmApsmApsmG-Chol-3'
106	5'-mGpsmApsmUpsmUpsmApsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmApsmApsmApsmG-GalNAc-3'
107	5'-mGpsmApsmUpsmUpsmApsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmApsmApsmApsmG
108	5'-mGpsmApsmUpsmUpsmApsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmApsmApsmApsmG-Chol-3'
109	5'-mGpsmApsmUpsmUpsmApsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmApsmApsmApsmG-GalNAc-3'

#ID	Modified Sequence (5'-3')
110	5'-mGpsmDAPpsmUpsmUpsmDAPpsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmApsmApsmApsmG-3'
111	5'-mGpsmApsmUpsmUpsmApsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmDAPpsmDAPpsmDAPpsmG-3'
112	5'-mGpsmApsmUpsmUpsmApsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmDAPpsmDAPpsmDAPpsmDAPpsmDAPpsmG-3'
113	5'-mGpsmDAPpsmUpsmUpsmDAPpsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmDAPpsmDAPpsmDAPpsmG-3'
114	5'-mGpsmDAPpsmUpsmUpsmDAPpsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmDAPpsmDAPpsmDAPpsmDAPpsmDAPpsmG-3'
115	5'-mGpsmDAPpsmUpsmUpsmDAPpsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmApsmApsmApsmG-3'
116	5'-mGpsmApsmUpsmUpsmApsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmDAPpsmDAPpsmDAPpsmG-3'
117	5'-mGpsmApsmUpsmUpsmApsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmDAPpsmDAPpsmDAPpsmDAPpsmDAPpsmG-3'
118	5'-mGpsmDAPpsmUpsmUpsmDAPpsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmDAPpsmDAPpsmDAPpsmG-3'
119	5'-mGpsmDAPpsmUpsmUpsmDAPpsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmDAPpsmDAPpsmDAPpsmDAPpsmDAPpsmG-3'
120	5'-mGpsmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmApsmDAPpsmGpsmUpsmGpsmC-3'
121	5'-mGpsmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmDAPpsmDAPpsmGpsmUpsmGpsmC-3'
122	5'-mGpsmCpsmApsmGpsmDAPpsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmDAPpsmDAPpsmGpsmUpsmGpsmC-3'
123	5'-mGpsmCpsmDAPpsmGpsmDAPpsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmDAPpsmDAPpsmGpsmUpsmGpsmC-3'
124	5'-CnpsGnpsTnpsGnpsCnpsApsGpsApsGpsGpsTpsGpsAnpsAnpsGnpsCnps-3-NH_2-G-3'
125	5'-GnpsCnpsAnpsGnpsAnpsGpsGpsTpsGpsApsApsGnpsCnpsGnpsAnpsAnps-3-NH_2-G-3
126	5'-CnpsGnpsAnpsCnpsGnpsTnpsGpsCpsApsGpsApsGpsGnpsTnpsGnpsAnpsAnpsGnps-3-NH_2-C-3'
127	5'-GnpsCnpsAnpsGnpsAnpsGnpsGpsTpsGpsApsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3-NH_2-C-3'
128	5'-GnpsCnpsAnpsGnpsApsGpsGpsTpsGpsAnpsAnpsGnpsCnps-3-NH_2-G-3'
129	5'-CnpsGnpsTnpsGnpsCnpsApsGpsApsGpsGpsTnpsGnpsAnpsAnpsGnps-3-NH_2-C-3'
130	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGpsApsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'
131	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTpsGpsAnpsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'
132	5'-GnpsCnpsAnpsGnpsAnpsGnpsGpsTpsGnpsAnpsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'
133	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGnpsApsApsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'
134	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGnpsAnpsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'
135	5'-GnpsCnpsAnpsGnpsAnpsGnpsGpsTpsGpsAnpsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'
136	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGpsApsApsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'
137	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTpsGpsApsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'
138	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGnpsApsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-

#ID	Modified Sequence (5'-3')
	C-3'
139	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGpsApsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'
140	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTpsGpsApsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'
141	5'-GnpsCnpsAnpsGnpsAnpsGnpsGpsTpsGpsApsApsGpsCpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'
142	5'-mApsmApsmGpsmApsmGpsApsGpsGpsTpsGps5mCpsGps5mCps5mCps5mCps5mmCpsmGpsmUpsmGpsmG-3'
143	5'-mGpsmGpsmUpsmGpsmApsApsGps5mCpsGpsApsApsGpsTpsGps5mCpsmAps5mmCpsmAps5mmCpsmG-3'
144	5'-5mmCpsmGpsmUpsmGps5mmCpsApsGpsApsGpsGpsTpsGpsApsApsGps5mmCpsmGpsmApsmApsmG-3'
145	5'-mApsmGpsmApsmGpsmGpsTpsGpsApsApsGps5mCpsGpsApsApsGpsmUpsmGps5mmCpsmAps5mmC-3'
146	5'-mUpsmGpsmGps5mmCpsmAps5mCpsTpsApsGpsTpsApsApsAps5mCpsTpsmGpsmApsmGps5mmCps5mmC-3'
147	5'-5mmCpsmUpsmApsmGpsmGpsApsGpsTpsTps5mCps5mCpsGps5mCpsApsGpsmUpsmApsmUpsmGpsmG-3'
148	5'-mApsmGpsmApsmGpsmGpsTpsGps5mCpsGps5mCps5mCps5mCps5mCpsGpsTpsmGpsmGpsmUps5mmCpsmG-3'
149	5'-mGpsmApsmGpsmGpsmUpsGps5mCpsGps5mCps5mCps5mCps5mCpsGpsTpsGpsmGpsmUps5mmCpsmGpsmG-3'
150	5'-mGpsmApsmApsmApsmGps5mCps5mCps5mCpsTpsAps5mCpsGpsApsAps5mCps5mmCpsmAps5mmCpsmUpsmG-3'
151	5'-mGpsmUpsmUps5mmCps5mmCpsGps5mCpsApsGpsTpsApsTpsGpsGpsApsmUps5mmCpsmGpsmGps5mmC-3'
152	5'-mUps5mmCps5mmCpsmGps5mmCpsApsGpsTpsApsTpsGpsGpsApsTps5mCpsmGpsmGps5mmCpsmApsmG-3'
153	5'-mAps5mmCps5mmCpsmAps5mmCpsTpsGpsApsAps5mCpsApsApsApsTpsGpsmGps5mmCpsmAps5mmCpsmU-3'
154	5'-mUpsmGps5mmCpsmApsmGpsApsGpsGpsTpsGpsApsApsGps5mCpsGpsmApsmApsmGpsmUpsmG-3'
155	5'-mAps5mmCpsmUpsmGpsmApsAps5mCpsApsApsApsTpsGpsGps5mCpsAps5mmCpsmUpsmApsmGpsmU-3'
156	5'-mApsmGpsmUps5mmCps5mmCpsAps5mCps5mCpsAps5mCpsGpsApsGpsTps5mCpsmUpsmApsmGpsmAps5mmC-3'
157	5'-5mmCpsmAps5mmCpsmUpsmGpsApsAps5mCpsApsApsApsTpsGpsGps5mCpsmAps5mmCpsmUpsmApsmG-3'
158	5'-5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5mCpsGpsApsApsmGpsmUpsmGps5mmCpsmA-3'
159	5'-mApsmApsmGpsmApsmGpsApsGpsGpsTpsGps5meCpsGps5meCps5meCps5meCps5memCpsmGpsmUpsmGpsmG-GalNAc-3'
160	5'-mGpsmGpsmUpsmGpsmApsApsGps5meCpsGpsApsApsGpsTpsGps5mCpsmAps5memCpsmAps5memeCps mG-GalNAc-3'
161	5'-mUpsmGpsmGps5memCpsmAps5meCpsTpsApsGpsTpsApsApsAps5meCpsTpsmGpsmApsmGps5memCps5memCpoGalNAc-3'
162	5'-5memCpsmUpsmApsmGmGpsApsGpsTpsTps5meCps5meCpsGps5meCpsApsGpsmUpsmApsmUpsmGpsmGpoGalNAc-3'
163	5'-mApsmGpsmApsmGpsmGpsTpsGps5meCpsGps5meCps5meCps5meCps5meCpsGpsTpsmGpsmGpsmUps 5memCpsmGpoGalNAc-3'
164	5'-mUps5memCps5memCpsmGps5memCpsApsGpsTpsApsTpsGpsGpsApsTps5meCpsmGpsmGps5memCps mApsmGpoGalNAc-3'
165	5'-mUpsmGps5memCpsmApsmGpsApsGpsGpsTpsGpsApsApsGps5meCpsGpsmApsmApsmGpsmUpsmG

#ID	Modified Sequence (5'-3')
	poGalNAc-3'
166	5'-mApsmGpsmUps5memCps5memCpsAps5meCps5meCAps5meCpsGpsApsGpsTps5meCpsmUpsmAps mGps mAps5memCpoGalNAc-3'
167	5'-fGnpsfCnpsfAnpsfGnpsfAnpsfGnpsGpsTpsGpsApsApsGpsfCnpsfGnpsfAnpsfAnpsfGnpsfUnpsfGnps-3-NH_2-fC-3'
168	5'-fGnpsfCnpsfAnpsfGnpsfAnpsfGnpsGpsTpsGpsApsApsGpsCpsfGnpsfAnpsfAnpsfGnpsfUnpsfGnps-3-NH_2-fC-3'
169	5'-fGnpsfCnpsfAnpsfGnpsfAnpsfGnpsGpsTpsGpsApsApsGpsCpsGpsfAnpsfAnpsfGnpsfUnpsfGnps-3-NH_2-fC-3'
170	5'-GnpCnpAnpGnpAnpGnpGpsTpsGpsApsApsGpsCnpGnpAnpAnpGnpTnpGnp-3 NH_2-C-3'
171	5'-GnpsfCnpsfAnpsGnpsfAnpsGnpsGpsTpsGpsApsApsGpsfCnpsGnpsfAnpsfAnpsGnpsfTnpsGnps-3 NH_2-fC-3'
172	5'-GnpfCnpfAnpGnpfAnpGnpGpsTpsGpsApsApsGpsfCnpGnpfAnpfAnpGnpfTnpGnp-3 NH_2-fC-3'
173	5'-GnpsafCnpsafAnpsGnspafAnpsGnpsGpsTpsGpsApsApsGpsafCnpsGnpsafAnpsafAnpsGnpsafUnpsGnps afC-3'
174	5'-GnpafCnpafAnpGnpafAnpGnpGpsTpsGpsApsApsGpsafCnpGnpafAnpafAnpGnpafUnpGnpafC-3'
175	5'-GnpafCnpafAnpGnpafAnpGnpGpsTpsGpsApsApsGpsCpsGnpafAnpafAnpGnpafUnpGnpafC-3'
176	5-mGpsmCpsmUpsmCpsmCpsmAps ApsApsTpsTpsCpsTpsTpsTpsApsmUpsmApsmApsmGpsmGpsmG-GalNAc-3
177	5'-mApsmApsmGpsmApsmGpsApsGpsGpsTpsGps5mCpsGps5mCps5mCps5mCps5mmCpsmGpsmUpsm GpsmG-3'
178	5'-mGpsmGpsmUpsmGpsmApsApsGps5mCpsGpsApsApsGpsTpsGps5mCpsmAps5mmCpsmAps5mmCps mG-3'
179	5-5mmCpsmGpsmUpsmGps5mmCpsApsGpsApsGpsGpsTpsGpsApsApsGps5mmCpsmGpsmApsmApsmG -3'
180	5'-mGpsmUpsmGpsmApsmApsGps5mCpsGpsApsApsGpsTpsGps5mCpsAps5mmCpsmAps5mmCpsmGps mG-3'
181	5'-mApsmGpsmApsmGpsmGpsTpsGpsApsApsGps5mCpsGpsApsApsGpsmUpsmGps5mmCpsmAps5mm C-3'
182	5'-mUpsmGpsmGps5mmCpsmAps5mCpsTpsApsGpsTpsApsApsAps5mCpsTpsmGpsmApsmGps5mmCps 5mmC-3'
183	5'-5mmCpsmUpsmApsmGpsmGpsApsGpsTpsTps5mCps5mCpsGps5mCpsApsGpsmUpsmApsmUpsmGps mG-3'
184	5'-mGps5mmCpsmApsmGpsmApsGpsGpsTpsGpsApsApsGps5mCpsGpsApsmApsmGpsmUpsmGps5mm C-3'
185	5'-mApsmGpsmApsmGpsmGpsTpsGps5mCpsGps5mCps5mCps5mCps5mCpsGpsTpsmGpsmGpsmUps5m mCpsmG-3'
186	5'-mGpsmApsmGpsmGpsmUpsGps5mCpsGps5mCps5mCps5mCps5mCpsGpsTpsGpsmGpsmUps5mmCps mGpsmG-3'
187	5'-mGpsmApsmApsmApsmGps5mCps5mCps5mCpsTpsAps5mCpsGpsApsAps5mCps5mmCpsmAps5mm CpsmUpsmG-3'
188	5'-mGpsmUpsmUps5mmCps5mmCpsGps5mCpsApsGpsTpsApsTpsGpsGpsApsmUps5mmCpsmGpsmGps 5mmC-3'
189	5'-mUps5mmCps5mmCpsmGps5mmCpsApsGpsTpsApsTpsGpsGpsApsTps5mCpsmGpsmGps5mmCpsmA psmG-3'
190	5'-mAps5mmCps5mmCpsmAps5mmCpsTpsGpsApsAps5mCpsApsApsApsTpsGpsmGps5mmCpsmAps5m mCpsmU-3'
191	5'-mUpsmGps5mmCpsmApsmGpsApsGpsGpsTpsGpsApsApsGps5mCpsGpsmApsmApsmGpsmUpsmG-3'
192	5'-mAps5mmCpsmUpsmGpsmApsAps5mCpsApsApsApsTpsGpsGps5mCpsAps5mmCpsmUpsmApsmGps mU-3'
193	5'-mApsmGpsmUps5mmCps5mmCpsAps5mCps5mCpsAps5mCpsGpsApsGpsTps5mCpsmUpsmApsmGps mAps5mmC-3'

#ID	Modified Sequence (5'-3')
194	5'-5mmCpsmAps5mmCpsmUpsmGpsApsAps5mCpsApsApsApsTpsGpsGps5mCpsmAps5mmCpsmUpsmApsmG-3'
195	5'-5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5mCpsGpsApsApsmGpsmUpsmGps5mmCpsmA-3'
196	5'-mApsmApsmGpsmApsmGpsmApsGpsGpsTpsGps5meCpsGps5meCps5meCps5memCps5memCpsmGpsmUpsmGpsmG 3'
197	5'-mApsmApsmGpsmApsmGpsmApsmGpsGpsTpsGps5meCpsGps5meCps5memCps5memCps5memCpsmGpsmUpsmGpsmG 3'
198	5'-mGpsmGpsmUpsmGpsmApsmApsGps5meCpsGpsApsApsGpsTpsGps5memCpsmAps5memCpsmAps5memeCpsmG 3'
199	5'-mGpsmGpsmUpsmGpsmApsmApsmGps5meCpsGpsApsApsGpsTpsGps5memCpsmAps5memCpsmAps5memeCpsmG 3'
200	5'-mUpsmGpsmGps5memCpsmAps5memCpsTpsApsGpsTpsApsApsAps5meCpsTpsmGpsmApsmGps5memCps5memC 3'
201	5'-mUpsmGpsmGps5memCpsmAps5meCpsTpsApsGpsTpsApsApsAps5meCpsTpsmGpsmApsmGps5memCps5memC 3'
202	5'-5memCpsmUpsmApsmGmGpsmApsGpsTpsTps5meCps5meCpsGps5meCpsApsmGpsmUpsmApsmUpsmGpsmG 3'
203	5'-5memCpsmUpsmApsmGmGpsmApsmGpsTpsTps5meCps5meCpsGps5meCpsApsmGpsmUpsmApsmUpsmGpsmG 3'
204	5'-GalNAc-NHC6-psmUpsm5meCpsm5meCpsmGpsm5meCpsApsGpsTpsApsTpsGpsGpsApsTps5meCpsmGpsmGpsm5meCpsmApsmG 3'
205	5'-GalNAc-NHC6-psm5meCpsmUpsmApsmGpsmGpsApsGpsTpsTps5meCps5meCpsGps5meCpsApsGpsmUpsmApsmUpsmGpsmG 3'
206	5'-GalNAc-NHC6-psmApsmApsmGpsmApsmGpsApsGpsGpsTpsGps5meCpsGps5meCps5meCps5meCpsm5meCpsmGpsmUpsmGpsmG 3'
207	5' GalNAc-NHC6-psmApsmGpsmApsmGpsmGpsTpsGps5meCpsGps5meCps5meCps5meCps5meCpsGpsTpsmGpsmGpsmUpsm5meCpsmG 3'
208	5' GalNAc-NHC6-psmUpsmGpsm5meCpsmApsmGpsApsGpsGpsTpsGpsApsApsGps5meCpsGpsmApsmApsmGpsmUpsmG 3'
209	mGpsmCpsmUpsmCpsmCpsmApsmApsApsTpsTpsCpsTpsTpsTpsmApsmUpsmApsmApsmGpsmG
210	mGpsmCpsmUpsmCpsmCpsmApsApsApsTpsTpsCpsTpsTpsTpsmApsmUpsmApsmApsmGpsmG
211	mGpsmCpsmUpsmCpsmCpsmApsApsApsTpsTpsCpsTpsTpsTpsApsmUpsmApsmApsmGpsmGpsmG
212	mGpsmCpsmUpsmCpsmCpsmApsmApsApsTpsTpsCpsTpsTpsTpsmApsmUpsmApsmApsmGpsmG/GalNAc/
213	mGpsmCpsmUpsmCpsmCpsmApsApsApsTpsTpsCpsTpsTpsTpsmApsmUpsmApsmApsmGpsmG/GalNAc/
214	mGpsmCpsmUpsmCpsmCpsmApsmApsApsTpsTpsCpsTpsTpsTpsmApsmUpsmApsmApsmGpsmG/3CholTEG/
215	mGpsmCpsmUpsmCpsmCpsmApsApsApsTpsTpsCpsTpsTpsTpsmApsmUpsmApsmApsmGpsmG/3CholTEG/
216	mGpsmCpsmUpsmCpsmCpsmApsApsApsTpsTpsCpsTpsTpsTpsApsmUpsmApsmApsmGpsmGpsmG/3CholTEG/
217	5'-mGps5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGp5mmCpsmGpsmApsmApsmGpsmUpsmGpsm5meC-3
218	5'-mGps5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5mmCpsmGpsmApsmApsmGpsmUpsmGps5mmC-Cholesterol-3'
219	5'-mGps5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGp5mmCpsmGpsmApsmApsmGpsmUpsmGp

#ID	Modified Sequence (5'-3')
	s5mmC-TEG-Cholesterol-3'
220	5'-mGps5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5mmCpsmGpsmApsmApsmGpsmUpsmGps5mmC-Tocopherol-3'
221	5'-mGps5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5mmCpsmGpsmApsmApsmGpsmUpsmGps5mmC-TEG-Tocopherol-3'
222	5'-mGps5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5mmCpsmGpsmApsmApsmGpsmUpsmGps5mmC-GalNAc-3'
223	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsmGpsmApsmApsmGpsmUpsmGpsm5meC-3'
224	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsmGpsmApsmApsmGpsmUpsmGpsm5meC-po-Chol-3'
225	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsmGpsmApsmApsmGpsmUpsmGpsm5meC-po-Tocopherol-3'
226	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsmGpsmApsmApsmGpsmUpsmGpsm5meC-po-GalNAc-3'
227	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsGpsmApsmApsmGpsmUpsmGpsm5meC-3'
228	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsGpsmApsmApsmGpsmUpsmGpsm5meC-po-Chol-3'
229	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsGpsmApsmApsmGpsmUpsmGpsm5meC-po-Tocopherol-3'
230	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsGpsmApsmApsmGpsmUpsmGpsm5meC-po-GalNAc-3'
231	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps(5m)mC-3
232	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps(5m)mC-Chol-3
233	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps(5m)mC-Toco-3
234	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps(5m)mC-GalNAc-3
235	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps(5m)mC-3
236	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps(5m)mC-Chol-3
237	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps(5m)mC-Toco-3
238	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps(5m)mC-GalNAc-3
239	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps(5m)mC-3
240	5-dTnpsGnpsCnpsAnpsGnpsApsGpsGpsTpsGpsApsApsGpsCpsGpsAnpsAnpsGnpsTnpsGn-3

#ID	Modified Sequence (5'-3')
241	5-dTnpsfGnpsCnpsfAnpsfGnpsApsGpsGpsTpsGpsApsApsGpsCpsGpsfAnpsfAnpsfGnpsfUnpsfGn 3'
242	5-fGnpsCnpsfAnpsfGnpsfAnpsfGnpsGpsTpsGpsApsApsGpsCnpsfGnpsfAnpsfAnpsfGnpsfUnpsfGnpsCn-3'
243	5-fGnpsCnpsfAnpsfGnpsfAnpsfGnpsGpsTpsGpsApsApsGpsCpsGpsfAnpsfAnpsfGnpsfUnpsfGnpsCn-3'
244	5'-dGnpmCnpmAnpmGnpmAnpmGnpGpsTpsGpsApsApsGpsmCnpmGnpmAnpmAnpmGnpmUnpmGnpmCnp-3'
245	5'-dGnpmCnpmAnpmGnpmAnpmGnpGpsTpsGpsApsApsGpsCpsmGnpmAnpmAnpmGnpmUnpmGnpmCnp-3'
246	5'-dGnpmCnpmAnpmGnpmAnpmGnpGpsTpsGpsApsApsGpsCpsGpsmAnpsmAnpmGnpmUnpmGnpmCnp-3'
247	5'-dGnpmCnpmAnpmGnpmAnpGpsGpsTpsGpsApsApsGpsCpsmGnpmAnpmAnpmGnpmUnpmGnpmCnp-3'
248	5'-dGnpmCnpmAnpmGnpmAnpGpsGpsTpsGpsApsApsGpsCpsGpsmAnpmAnpmGnpmUnpmGnpmCnp-3'
249	GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGnpsAnpsAnpsGnpsCnpsGnpsAnpsAnpsG
250	GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGnpsAnpsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnpsC
251	CnpsGnpsTnpsGnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGnpsAnpsAnpsGnpsCnpsG
252	CnpsGnpsTnpsGnpsCnpsApsGpsApsGpsGpsTpsGpsAnpsAnpsGnpsCnpsG
253	GnpsCnpsAnpsGnpsAnpsGpsGpsTpsGpsApsApsGnpsCnpsGnpsAnpsAnpsG
254	CnpsGnpsAnpsCnpsGnpsTnpsGpsCpsApsGpsApsGpsGnpsTnpsGnpsAnpsAnpsGnpsC
255	GnpsCnpsAnpsGnpsAnpsGnpsGpsTpsGpsApsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnpsC
256	GnpsCnpsAnpsGnpsApsGpsGpsTpsGpsAnpsAnpsGnpsCnpsG
257	CnpsGnpsTnpsGnpsCnpsApsGpsApsGpsGpsTnpsGnpsAnpsAnpsGnpsC
258	mGnpsmoeCnpsmoeAnpsmGnpsmoeAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmoeAnpsmGnpsmoeUnpsmGnpsmoeCnp-C6-NH-GalNAc6
259	moeGps(5me)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsApsGps(5me)CpsGpsApsmoeApsmoeGpsmoeTpsmoeGps(5me)moeC-po-GalNAc2
260	mGnpsmoeCnpsmoeAnpsmGnpsmoeAnpsGpsGpsTpsGpsApsApsGps(5me)CpsGpsApsmoeAnpsmGnpsmoeUnpsmGnpsmoeCnpo-C6-NH-GalNAc-6
261	GalNAc-2-pofGnpsfCnpsfAnpsfGnpsfAnpsGpsGpsTpsGpsApsApsGps(5me)CpsGpsApsfAnpsfGnpsfUnpsfGnpsfCn
262	GalNAc2-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn
263	moeGps(5me)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsApsGps(5me)CpsGpsApsmoeApsmoeGpsmoeTpsmoeGps(5me)moeC-GalNAc2
264	GalNac2-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn
265	GalNac-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn
266	GalNAc2-mGnpsmCnpsmAnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn
267	GalNac6- NH-C6-moeGps(5m)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeApsmoeGps

#ID	Modified Sequence (5'-3')
	moeTpsmoeGps(5m)moeC
268	GalNAc2-mGnpsmCnpsmAnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmAnpsmGnpsmUnpsmGnpsmCn
269	GalNAc2-etoGnps(5m)etoCnpsetoAnpsetoGnpsetoAnpsGps GpsTpsGpsApsApsGps(5m)CpsGpsApsetoAnpsetoGnpsetoTnpsetoGnps(5m)etoCn
270	GalNAc2-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmAnpsmGnpsmUnpsmGnpsmCn
271	moeGpsmoemCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsApsGps5mCpsGpsApsmoeApsmoeGpsmoeTpsmoeGpsmoemC
272	moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn
273	mGps5mmCpsmApsmGpsmApsGpsGpsTpsGpsApsApsGps5mCpsGpsApsmApsmGpsmUpsmGps5mmC
274	mGnpsmCnpsmAnpsmGnps mAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmAnpsmGnpsmUnpsmGnpsmCn
275	GalNAc2-moeGnpsmoeCnpsmoeAnps moeGnpsmoeAnpsGps GpsTpsGps ApsApsGps (5m)CpsGpsAps moeAnpsmoeGnpsmoeUnps moeGnpsmoeCn
276	GalNac6-NH-C6-moeGps(5m)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeApsmoeGps moeTpsmoeGps(5m)moeC
277	5'moeGps(5m)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeApsmoeGpsmoeTpsmoeGps(5me)moeC-GalNAc2
278	GalNAc2-mGnpsmCnpsmAnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmAnpsmGnpsmUnpsmGnpsmCn'
279	mGps(5m)mCpsmApsmGpsmApsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmApsmGpsmUpsmGps(5m)mC-GalNAc
280	GalNAc2-moeGnpsmoeCnpsmoeAnps moeGnpsmoeAnpsGpsGpsTpsGpsApsApsGps (5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnps moeGnpsmoeCn
281	moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCnp-C6-NH-GalNAc6
282	GalNAc2-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnps moeGnpsmoeCn
283	GalNAc-GnpsCnpsAnpsGnpsAnpsGps GpsTpsGpsApsApsGpsCpsGpsApsAnpsGnpsTnpsGnpsCn
284	GalNAc-mGnpsmCnpsmAnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmAnpsmGnpsmUnpsmGnpsmCn
285	GalNAc-fGnpsfCnpsfAnpsfGnpsfAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsfAnpsfGnpsfUnpsfGnps-3nh2-fC
286	GalNAc-afGnpsafCnpsafAnpsafGnpsafAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsafAnpsafGnpsafTnpsafGnpsafCn
287	GalNAc-dTnpsGnpsCnpsAnpsGnpsApsGpsGpsTpsGpsApsApsGpsCpsGpsAnpsAnpsGnpsTnps-3nh2-G
288	GalNAc-mUnpsmGnpsmCnpsmAnpsmGnpsApsGpsGpsTpsGpsApsApsGpsCps GpsmAnpsmAnpsmGnpsmUnpsmGn

#ID	Modified Sequence (5'-3')
289	GalNAc-fUnpsfGnpsfCnpsfAnpsfGnpsApsGpsGpsTpsGpsApsApsGpsCpsGpsfAnpsfAnpsfGnpsfUnps-3nh2-fG
290	GalNAc-mGnpsmCnpsmUnpsmCnpsmCnpsApsApsApsTpsTps5MeCpsTpsTpsTpsApsmUnpsmAnpsmAnpsmGnpsmGnpsmGn
291	GalNAc-moeGnpsmoeCnpsmoeUnpsmoeCnpsmoeCnpsApsApsApsTpsTps5MeCpsTpsTpsTpsApsmoeUnpsmoeAnpsmoeAnpsmoeGnpsmoeGnpsmoeGn
292	moeGps(5m)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeApsmoeGpsmoeTpsmoeGps(5m)moeC
293	moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn
294	fGnps(5m)fCnpsfAnpsfGnpsfAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsfAnpsfGnpsfTnpsfGnpsfC-C6-NH-GalNac6
295	fGnpsfCnpsfAnpsfGnpsfAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsfAnpsfGnpsfUnpsfGnpsfCnp-C6-NH-GalNAc6
296	mGnpsmCnpsmAnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsAps mAnpsmGnpsmUnps mGnpsmCnp-C6-NH-GalNAc6
297	mGnpsmCnpsmAnpsmGnpsmAnpsGpGpsTpsGpsApsApsGps(5m)CpsGpsAps mAnpsmGnpsmUnpsmGnpsmC-C6-NH-GalNAc6
298	moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGps GpsTpsGps ApsApsGps (5m)CpsGpsAps moeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCnp-C6-NH-GalNAc6
299	moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnps(5m)moeC-C6-NH-GalNAc6
300	GalNAc2-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGps ApsApsGps (5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn
301	GalNAc2-etoGnpseto(5m)CnpsetoAnps etoGnpsetoAnpsGpsGpsTpsGpsApsApsGps (5m)CpsGpsAps etoAnpsetoGnpsetoTnps etoGnpseto(5m)Cn
302	mGnpsmCnps2-4-OCH_2AnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsAps2-4-OCH_2AnpsmGnpsmUnpsmGnps3-NH_2mC
303	mGnpsmCnps2-4-OCH_2CH_2AnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsAps2-4-OCH_2CH_2AnpsmGnpsmUnpsmGnps3-NH_2mC
304	mGnpsmCnps2-4-OCH_2CH_2AnpsmGnps2-4OCH_2CH_2AnpsGpsGpsTpsGpsApsApsGpsCpsGpsAps2-4-OCH_2CH_2AnpsmGnpsmUnpsmGnps3-NH_2mC
305	mGnpsmCnpsmAnpsmGnps2-4-OCH_2CH_2AnpsGpsGpsTpsGpsApsApsGpsCpsGpsAps2-4-OCH_2CH_2AnpsmGnpsmUnpsmGnps3-NH_2mC
306	5-mGnpsmCnpsmUnpsmCnpsmCnps2-4-OCH_2CH_2AnpsApsApsTpsTpsCpsTpsTpsTps mAnpsmUnpsmAnps2-4-OCH_2CH_2AnpsmGnpsmGnps3-NH_2mG-3
307	mGnpsmCnpsmUnpsmCnpsmCnps2-4-OCH_2CH_2AnpsApsApsTpsTpsCpsTpsTpsTps2-4-OCH_2CH_2AnpsmUnpsmAnps2-4-OCH_2 CH_2AnpsmGnpsmGnps3-NH_2mG
308	mGnpsmCnpsmUnpsmCnpsmCnps2-4-OCH_2CH_2AnpsApsApsTpsTpsCpsTpsTpsTps2-4-OCH_2 CH_2AnpsmUnps2-4-OCH_2CH_2AnpsmAnpsmGnpsmGnps3-NH_2mG
309	2-4OCH_2CH_2GnpsmCnpsmAnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmAnpsmGnpsmUnps2-4OCH_2CH_2Gnps3-NH_2mC
310	2-4 OCH_2CH_2GnpsmCnpsmAnps2-4OCH_2CH_2GnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmAnps2-4 OCH_2CH_2GnpsmUnps2-4OCH_2CH_2Gnps3-NH_2mC
311	2-4OCH_2CH_2GnpsmCnps2-4-OCH_2CH_2AnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmAnpsmGnpsmUnpsmGnps3-NH_2mC
312	2-4OCH_2CH_2GnpsmCnpsmUnpsmCnpsmCnps2-4-OCH_2CH_2AnpsApsApsTpsTpsCpsTpsTpsTpsmAnpsmUnpsmAnps2-4-OCH_2CH_2AnpsmGnpsm2-

#ID	Modified Sequence (5'-3')
	4OCH_2CH_2Gnps3-NH_2mG
313	2-4 OCH_2CH_2GnpsmCnpsmUnpsmCnpsmCnpsmAnpsApsApsTpsTpsCpsTpsTpsTps mAnpsmUnpsmAnpsmAnpsmGnpsm2-4 OCH_2CH_2Gnps3-NH_2mG
314	mGnpsmCnpsmAnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmAnpsmGnpsmUnpsmGnps3-NH_2mC
315	mGnps2-4 OCH_2CH_2(5me)CnpsmAnps2-4 OCH_2CH_2GnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmAnpsmGnps2-4 OCH_2CH_2TnpsmGnps3-NH_2mC
316	2-4 OCH_2CH_2GnpsmCnps2-4OCH_2CH_2AnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmAnpsmGnps2-4 OCH_2CH_2TnpsmGnps2-$OCH_2CH_2$3-NH_2(5me)C
317	2-4 OCH_2CH_2GnpsmCnps2-4 OCH_2CH_2TnpsmCnpsmCnpsmAnpsApsApsTpsTpsCpsTpsTpsTpsmAnps2-4 OCH_2CH_2TnpsmAnpsmAnpsmGnps2-4 OCH_2CH_2Gnps3-NH_2mG
318	mGnps2-4OCH_2CH_2(5me)CnpsmUnps2-4 OCH_2CH_2 (5me)CnpsmCnpsmAnpsApsApsTpsTpsCpsTpsTpsTpsmAnps2-4 OCH_2CH_2TnpsmAnpsmAnps2-4OCH_2CH_2GnpsmGnps3-NH_2mG

[0092] In some embodiments, the oligonucleotide represented by Formula (VI) or (VI') is selected from the above Table C. In other embodiments, the oligonucleotide represented by Formula (VI) or (VI') has a sequence that differs from a chimeric oligonucleotide of the above list by one nucleotide. In other embodiments, the oligonucleotide represented by Formula (VI) or (VI') has a sequence that differs from a chimeric oligonucleotide of the above list by 1, 2, 3 or 4 nucleotides. In embodiments, the oligonucleotide represented by Formula (VI) or (VI') has a sequence that differs from a chimeric oligonucleotide of the above list but has the same construct as the chimeric oligonucleotide of the above list. In embodiments, the disclosed oligonucleotides display an increased affinity for a target nucleic acid sequence compared to an unmodified oligonucleotide of the same sequence. For example, in some sequences the disclosed oligonucleotides has a nucleobase sequence that is complementary or hybridizes to a target nucleic acid sequence at a higher affinity than an unmodified oligonucleotide of the same sequence. In embodiments, the disclosed oligonucleotide complexed with a complementary target nucleic acid sequence has a melting temperature ™ of >37 °C. The complex may be formed under physiological conditions or nearly physiological conditions such as in phosphate-buffered saline (PBS). In embodiments, the Tm of the complex is >50 °C. In embodiments, the Tm of the complex is 50-100 °C. In embodiments, the Tm of a disclosed oligonucleotide duplexed with a target nucleic acid sequence under physiological conditions or nearly physiological conditions is >50 °C.

[0093] In certain embodiments, the target nucleic acid sequence may be selected from a nucleic acid sequence of a known viral DNA or RNA sequence such as the HBV genome, for example those listed in Table E, F, or J.

[0094] In embodiments, the disclosed oligonucleotides display an affinity for at least one of the following six sequences of the HBV genome or its RNA equivalents and/or display stability complexed to at least one of the following six sequences of the HBV genome (Table E) or its RNA equivalents (Table F). In embodiments, the oligonucleotide complexed with a complementary HBV genome sequence has a melting temperature (Tm) of >37 °C. The HBV genome may be an RNA sequence such as DR-1 and/or DR-2 RNA sequence. The complex may be formed under physiological conditions or nearly physiological conditions such as in phosphate-buffered saline (PBS). In embodiments, the Tm of the complex is >50 °C. In embodiments, the Tm of the complex is 50-100 °C. In embodiments, the Tm of a disclosed oligonucleotide duplexed with an HBV RNA under physiological conditions or nearly physiological conditions is >50 °C.

Table E

1		2		3		4		5		6	
245	A	668	T	1257	T	1512	A	1575	C	1819	A
246	G	669	G	1258	C	1513	C	1576	C	1820	C
247	T	670	G	1259	T	1514	C	1577	G	1821	T
248	C	671	C	1260	G	1515	G	1578	T	1822	T
249	T	672	T	1261	C	1516	A	1579	G	1823	T
250	A	673	C	1262	C	1517	C	1580	T	1824	T
251	G	674	A	1263	G	1518	C	1581	G	1825	T
252	A	675	G	1264	A	1519	A	1582	C	1826	C
253	C	676	T	1265	T	1520	C	1583	A	1827	A
254	T	677	T	1266	C	1521	G	1584	C	1828	C
255	C	678	T	1267	C	1522	G	1585	T	1829	C
256	G	679	A	1268	A	1523	G	1586	T	1830	T
257	T	680	C	1269	T	1524	G	1587	C	1831	C
258	G	681	T	1270	A	1525	C	1588	G	1832	T
259	G	682	A	1271	C	1526	G	1589	C	1833	G
260	T	683	G	1272	T	1527	C	1590	T	1834	C
261	G	684	T	1273	G	1528	A	1591	T	1835	C
262	G	685	G	1274	C	1529	C	1592	C	1836	T
263	A	686	C	1275	G	1530	C	1593	A	1837	A

264	C	687	C	1276	G	1531	T	1594	C	1838	A
265	T	688	A	1277	A	1532	C	1595	C	1839	T
266	T	689	T	1278	A	1533	T	1596	T	1840	C
		690	T	1279	C	1534	C	1597	C	1841	A
		691	T	1280	T	1535	T	1598	T	1842	T
		692	G	1281	C	1536	T	1599	G	1843	C
		693	T	1282	C	1537	T	1600	C	1844	T
		694	T	1283	T			1601	A	1845	C
		695	C	1284	A			1602	C	1846	T
		696	A	1285	G			1603	G	1847	T
		697	G	1286	C			1604	T	1848	G
		698	T					1605	C	1849	T
		699	G					1606	G	1850	T
		700	G					1607	C	1851	C
		701	T					1608	A	1852	A
		702	T					1609	T		
		703	C					1610	G		
		704	G					1611	G		
		705	T					1612	A		
		706	A								
		707	G								
		708	G								
		709	G								
		710	C								
		711	T								
		712	T								
		713	T								
		714	C								
		715	C								

Table F

1		2		3		4		5		6	
245	A	668	U	1257	U	1512	A	1575	C	1819	A
246	G	669	G	1258	C	1513	C	1576	C	1820	C
247	U	670	G	1259	U	1514	C	1577	G	1821	U
248	C	671	C	1260	G	1515	G	1578	U	1822	U
249	U	672	U	1261	C	1516	A	1579	G	1823	U
250	A	673	C	1262	C	1517	C	1580	U	1824	U
251	G	674	A	1263	G	1518	C	1581	G	1825	U
252	A	675	G	1264	A	1519	A	1582	C	1826	C
253	C	676	U	1265	U	1520	C	1583	A	1827	A

254	U	677	U	1266	C	1521	G	1584	C	1828	C
255	C	678	U	1267	C	1522	G	1585	U	1829	C
256	G	679	A	1268	A	1523	G	1586	U	1830	U
257	U	680	C	1269	U	1524	G	1587	C	1831	C
258	G	681	U	1270	A	1525	C	1588	G	1832	U
259	G	682	A	1271	C	1526	G	1589	C	1833	G
260	U	683	G	1272	U	1527	C	1590	U	1834	C
261	G	684	U	1273	G	1528	A	1591	U	1835	C
262	G	685	G	1274	C	1529	C	1592	C	1836	U
263	A	686	C	1275	G	1530	C	1593	A	1837	A
264	C	687	C	1276	G	1531	U	1594	C	1838	A
265	U	688	A	1277	A	1532	C	1595	C	1839	U
266	U	689	U	1278	A	1533	U	1596	U	1840	C
		690	U	1279	C	1534	C	1597	C	1841	A
		691	U	1280	U	1535	U	1598	U	1842	U
		692	G	1281	C	1536	U	1599	G	1843	C
		693	U	1282	C	1537	U	1600	C	1844	U
		694	U	1283	U			1601	A	1845	C
		695	C	1284	A			1602	C	1846	U
		696	A	1285	G			1603	G	1847	U
		697	G	1286	C			1604	U	1848	G
		698	U					1605	C	1849	U
		699	G					1606	G	1850	U
		700	G					1607	C	1851	C
		701	U					1608	A	1852	A
		702	U					1609	U		
		703	C					1610	G		
		704	G					1611	G		
		705	U					1612	A		
		706	A								
		707	G								
		708	G								
		709	G								
		710	C								
		711	U								
		712	U								
		713	U								
		714	C								
		715	C								

[0095] Compounds of the present disclosure include compounds comprising the following Formula (VII):

5'-X'—Y'—Z'-3' (VII)

wherein X'—Y'—Z' is a chimeric oligonucleotide comprising a sequence of 14 to 22 nucleosides, and is optionally conjugated at the 5' and/or 3' end to a ligand targeting group or a pharmacophore, X' is a domain comprising a sequence of modified nucleosides that is 3-14 nucleosides in length; Y' is a domain comprising a sequence of 2 to 4 2'-deoxynucleosides linked through intersubunit linkages; and Z' is a domain comprising a sequence of modified nucleosides that is 3-14 nucleosides in length, wherein the X' and/or Y' domains comprise one or more modified nucleoside which is linked through a N3′→P5′ phosphoramidate or a N3′→P5′ thiophosphoramidate intersubunit linkage.

[0096] The chimeric oligonucleotide represented by X'—Y'—Z' of Formula (VII) comprises a sequence of 14 to 22 nucleotides, for example, 14, 15, 16, 17, 18, 19, 20, 21, or 22 nucleotides. In some embodiments, the number of nucleotides in each of X', Y' and Z', respectively is: 8/2/10, 9/2/10, 10/2/10, 7/3/10, 8/3/10, 9/3/10, 8/4/8, 9/4/9, 6/4/8. In some embodiments, X' is 6-10, Y' is 2-4 and Z' is 8-10.

[0097] In some embodiments, the compound of Formula (VII) consists of the X'—Y'—Z' chimeric oligonucleotide consisting of a sequence of 14 to 22 nucleotides, and is optionally conjugated at the 5' and/or 3' end (e.g., 5' end, 3' end or both 5' and 3' ends) to a ligand targeting group and/or a pharmacophore, where X' is a domain consisting of a sequence containing one or more modified nucleotides that is 3-10 nucleotides in length; Z' is a domain consisting of a sequence containing one or more modified nucleotides that is 3-10 nucleotides in length; and Y' is a domain consisting of a sequence of 2 to 42'-deoxy-nucleotides linked through thiophosphate intersubunit linkages and optionally one phosphodiester intersubunit linkage, wherein the X' and/or Y' domains contain one or more modified nucleotide which is linked through a N3′→P5′ phosphoramidate or a N3′→P5′ thiophosphoramidate intersubunit linkage.

[0098] The X' domain comprises a sequence of modified nucleotides, where the X' domain is 4-10 nucleotides in length. For example, the X' domain may comprise a sequence of 4, 5, 6, 7, 8, 9, or 10 nucleotides. One or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19,

20, 21 or 22) of these nucleotides is modified. For example, in some embodiments, all the nucleotides in the X' domain are modified.

[0099] The modified nucleotides of the X' domain may be the same as disclosed for X in Formula (VI) or (VI'). For example, the nucleotides of the X' domain may be modified with respect to one or more of their nucleobases, the 2' and/or 3' positions on the ribose sugar and their intersubunit linkages. Embodiments include wherein the 2' position is modified with an F (ribo or arabino) and the 3' position is O or NH. Embodiments also include wherein the 2' position is modified with an OMe and the 3' position is O or NH. Embodiments include wherein the 2' position is modified with an F (ribo or arabino) as well as Me or OMe, and the 3' position is O or NH. Embodiments include wherein the 2' position is modified with an F (ribo or arabino) and the 3' position is O or NH. Embodiments include wherein the 2' position is modified with an O-methoxyethoxy and the 3' position is O or NH. Embodiments also include wherein the 2' position is modified with an F (ribo or arabino) and the 3' position is O or NH. Embodiments include wherein the 2' and 4' positions are modified bridging group (as described elsewhere herein) to form a conformationally restricted nucleotide and the 3' position is O or NH. Each of these embodiments may include thiophosphate (or thiophosphoramidate depending on the 3' substitution) and phosphoramidate intersubunit linkages.

[0100] Embodiments also include where the 2' position is OH, and the 3' position is NH, or where the 2' position is H, and the 3' position is NH. Each of these embodiments may include thiophosphoramidate and/or phosphoramidate intersubunit linkages.

[0101] The nucleotides of the X' domain are linked through intersubunit linkages, for example, N3'→P5' phosphoramidate, N3'→P5' thiophosphoramidate, thiophosphate or phosphodiester intersubunit linkages. In some embodiments, the X' domain is linked through intersubunit linkages selected from N3'→P5' phosphoramidate, N3'→P5' thiophosphoramidate, and combinations thereof. In some embodiments, the X' domain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 from N3'→P5' phosphoramidate and/or N3'→P5' thiophosphoramidate intersubunit linkages.

[0102] The Y' domain comprises a sequence of 2 to 4 2'-deoxynucleotides. For example, the Y' domain may comprise a sequence of 2, 3, or 4 2'-deoxynucleotides. One or more of the 2'-deoxynucleotides may be linked through thiophosphate or phosphodiester intersubunit linkages

(e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22). In some embodiments, each of the 2'-deoxynucleotides is linked through a thiophosphate intersubunit linkage. In other embodiments, each of the 2'-deoxynucleotides is linked through a phosphodiester intersubunit linkage. In other embodiments, the Y' domain consists of 2'-deoxy-nucleotides linked through thiophosphate intersubunit linkages, and optionally one phosphodiester intersubunit linkage.

[0103] The Z' domain comprises a sequence of modified nucleotides, where the Z' domain is 4-10 nucleotides in length. For example, the Z' domain may comprise a sequence of 4, 5, 6, 7, 8, 9, or 10 nucleotides. One or more of these nucleotides is modified (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22). For example, in some embodiments, all the nucleotides in the Z' domain are modified.

[0104] The modified nucleotides of the Z' domain may be the same as disclosed for Z in Formula (VI) or (VI'). For example, the nucleotides of the Z' domain may be modified with respect to one or more of their nucleobases, the 2' and/or 3' positions on the ribose sugar and their intersubunit linkages. Embodiments include wherein the 2' position is modified with an F (ribo or arabino) and the 3' position is O or NH. Embodiments also include wherein the 2' position is modified with an OMe and the 3' position is O or NH. Embodiments include wherein the 2' position is modified with an F (ribo or arabino) as well as Me or OMe, and the 3' position is O or NH. Embodiments include wherein the 2' position is modified with an F (ribo or arabino) and the 3' position is O or NH. Embodiments include wherein the 2' position is modified with an O-methoxyethoxy and the 3' position is O or NH. Embodiments also include wherein the 2' position is modified with an F (ribo or arabino) and the 3' position is O or NH. Embodiments include wherein the 2' and 4' positions are modified bridging group (as described elsewhere herein) to form a conformationally restricted nucleotide and the 3' position is O or NH. Each of these embodiments may include thiophosphate (or thiophosphoramidate depending on the 3' substitution) and phosphoramidate intersubunit linkages.

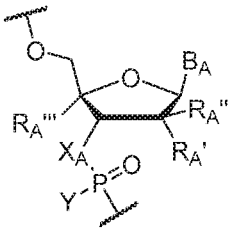
[0105] Embodiments also include where the 2' position is OH, and the 3' position is NH, or where the 2' position is H, and the 3' position is NH. Each of these embodiments may include thiophosphoramidate and/or phosphoramidate intersubunit linkages.

[0106] The nucleotides of the Z' domain are linked through intersubunit linkages, for example, N3′→P5′ phosphoramidate, N3′→P5′ thiophosphoramidate, thiophosphate or phosphodiester intersubunit linkages. In some embodiments, the Z' domain is linked through intersubunit linkages selected from N3′→P5′ phosphoramidate, N3′→P5′ thiophosphoramidate, and combinations thereof. In some embodiments, the Z' domain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 from N3′→P5′ phosphoramidate and/or N3′→P5′ thiophosphoramidate intersubunit linkages.

C. Modified Antisense Oligonucleotides

[0107] Other compounds include modified antisense oligonucleotides. In some embodiments the ASO includes the nucleotide of formula (I), (II), (IIIa), (IIIb), (IV), (V) and/or (V').

[0108] Other compounds of the present disclosure include compounds comprising the following Formula (VIII):



(VIII),

wherein X_A is NH or O, Y is OR or SR, where R is H or a positively charged counter ion, B_A is independently in each instance a natural or an unmodified nucleobase or a modified nucleobase, R_A' and R_A'' are each independently in each instance selected from H, F, OH, OMe, O-methoxyethoxy, and R_A''' is H or R_A' and R_A''' together form –O-CH_2–, –O-CH(Me)– or –O-$(CH_2)_2$–.

[0109] In some embodiments, R_A' and R_A''' are H; and R_A'' is selected from F, OH, OMe, Me, O-methoxyethoxy. In other embodiments, R_A'' and R_A''' are H; and R_A' is selected from F, OMe, Me, O-methoxyethoxy. In some embodiments, X_A is NH in each instance.

[0110] Some embodiments include one or more modified nucleotides represented by Formula (VIII), wherein X_A is NH; B_A is a G-clamp; R_A' is F or OMe and R_A'' is H; or R_A' is H and R_A'' is H or F; and R_A''' is H.

[0111] Some embodiments include one or more modified nucleotides represented by Formula (VIII), wherein X_A is NH; B_A is an unmodified or modified nucleobase; R_A' and R_A''' together form a conformationally restricted nucleotide (e.g., –O-CH_2– or –O-$(CH_2)_2$–); and R_A'' is H. In some embodiments, B_A is an unmodified or a modified nucleobase selected from the group consisting of 5-methylcytosine, 2,6-diaminopurine, and 5-methyluracil.

[0112] Some embodiments include one or more modified nucleotides represented by Formula (VIII), wherein X_A is NH; B is an unmodified or modified nucleobase; R_A' is F or OMe, R_A'' is H and R_A''' is H.

[0113] Some embodiments include one or more modified nucleotides represented by Formula (VIII), wherein X_A is NH; B_A is an unmodified or modified nucleobase; R_A' is H, R_A'' is F and R_A''' is H.

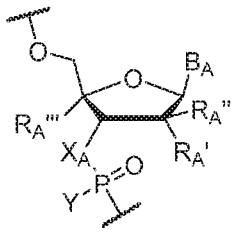
[0114] In some embodiments, X_A is NH. In other embodiments, Y is O^- or S^- (with a positively charged counter ion). In some embodiments, R_A' or R_A'' is H and the other is F, OH, OMe, Me, O-methoxyethoxy (e.g. arabino-F or ribo-F or OMe).

[0115] In some embodiments, B_A is selected from A, C, G, U and T. In additional embodiments, B_A is selected from A, C, G, U, T, 2,6-diaminopurine, a 5-Me pyrimidine (e.g., 5-methylcytosine, 5-methyluracil). In some embodiments, at least one of R_A' and R_A'' is H. For example, in some embodiments, R_A' is F, OH, OMe, Me, O-methoxyethoxy and R_A'' is H. In other embodiments, R_A' is H and R_A'' is F.

[0116] In some embodiments, when B_A is a purine nucleobase at least one of R_A' and R_A'' is OH or F, and/or when B_A is a pyrimidine nucleobase at least one of R_A' and R_A'' is OMe, OH or F.

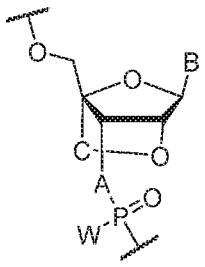
[0117] In other embodiments, the nucleotides include one or more of the nucleotides in Table G.

Table G



Nucleotide No.	R'	R''	R'''	A	W

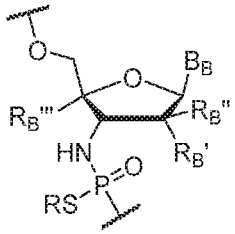
Nucleotide No.	R'	R''	R'''	A	W
48	F	H	H	NH	S
49	F	H	H	NH	O
50	F	H	H	O	S
51	F	H	H	O	O
52	H	F	H	NH	S
53	H	F	H	NH	O
54	H	F	H	O	S
55	H	F	H	O	O
56	OMe	H	H	NH	S
57	OMe	H	H	NH	O
58	OMe	H	H	O	S
59	OMe	H	H	O	O
60	H	F	H	NH	S
61	H	F	H	NH	O
62	H	F	H	O	S
63	H	F	H	O	O
64	O-methoxyethoxy	H	H	NH	S
65	O-methoxyethoxy	H	H	NH	O
66	O-methoxyethoxy	H	H	O	S
67	O-methoxyethoxy	H	H	O	O
68	H	H	H	NH	S
69	H	H	H	NH	O
70	OH	H	H	NH	S
71	OH	H	H	NH	O
72	OH	H	H	O	S
73	H	OH	H	NH	O
74	H	OH	H	NH	S
75	H	OEt	H	NH	O
76	H	OEt	H	NH	S
77	H	OEt	H	O	O
78	H	OEt	H	O	S
79	OEt	H	H	NH	O
80	OEt	H	H	NH	S
81	OEt	H	H	O	O
82	OEt	H	H	O	S



Nucleotide No.	C	A	W
83	-O-CH_2-	NH	S
84	-O-CH_2-	NH	O

Nucleotide No.	C	A	W
85	-O-CH_2-	O	S
86	-O-CH_2-	O	O
87	-O-$(CH_2)_2$-	NH	S
88	-O-$(CH_2)_2$-	NH	O
89	-O-$(CH_2)_2$-	O	S
90	-O-$(CH_2)_2$-	O	O
91	-O-CH(Me)-	NH	S
92	-O-CH(Me)-	NH	O
93	-O-CH(Me)-	O	S
94	-O-CH(Me)-	O	O

[0118] Compounds of the present disclosure also include oligonucleotides comprising ten or more nucleotides of the following Formula (IX):



(IX),

wherein R is H or a positively charged counter ion, B_B is independently in each instance a natural or an unmodified nucleobase or a modified nucleobase, R_B' and R_B'' are each independently in each instance selected from H, F, OMe, O-methoxyethoxy, and R_B''' is H or R_B' and R_B''' together form –O-CH_2–, –O-CH(Me)–, or –O-$(CH_2)_2$–.

[0119] In some embodiments, every oligonucleotide is a nucleotide of the Formula (IX).

[0120] In some embodiments, R_B' and R_B''' are H and R_B'' is selected from F, OH, OMe, Me, O-methoxyethoxy. In other embodiments, R_B'' and R_B''' are H; and R_B' is selected from F, OMe, Me, O-methoxyethoxy.

[0121] Some embodiments include one or more modified nucleotides represented by Formula (IX), wherein B_A is a G-clamp; R_B' is F or OMe and R_B'' is H; or R_B' is H and R_B'' is H or F; and R_B''' is H.

[0122] Some embodiments include one or more modified nucleotides represented by Formula (IX), wherein B_A is an unmodified or modified nucleobase; R_B' and R_B''' together form a conformationally restricted nucleotide (e.g., –O-CH_2– or –O-$(CH_2)_2$–); and R_B'' is H. In some embodiments, B_A is an unmodified or a modified nucleobase selected from the group consisting of 5-methylcytosine, 2,6-diaminopurine, and 5-methyluracil.

[0123] Some embodiments include one or more modified nucleotides represented by Formula (IX), wherein B is an unmodified or modified nucleobase; R_B' is F or OMe, R_B'' is H and R_B''' is H.

[0124] Some embodiments include one or more modified nucleotides represented by Formula (IX), wherein B_A is an unmodified or modified nucleobase; R_B' is H, R_B'' is F and R_B''' is H.

[0125] In other embodiments, Y is S^- (with a positively charged counter ion). In some embodiments, R_B' or R_B'' is H and the other is F, OH, OMe, Me, O-methoxyethoxy (e.g. arabino-F or ribo-F or OMe).

[0126] In some embodiments, B_B is selected from A, C, G, U and T. In additional embodiments, B_B is selected from A, C, G, U, T, 2,6-diaminopurine, a 5-Me pyrimidine (e.g., 5-methylcytosine). In some embodiments, at least one of R_B' and R_B'' is H. For example, in some embodiments, R_A' is F, OH, OMe, Me, O-methoxyethoxy and R_B'' is H. In other embodiments, R_B' is H and R_B'' is F.

[0127] In some embodiments, when B_B is a purine nucleobase at least one of R_B' and R_B'' is OH or F, and/or when B_B is a pyrimidine nucleobase at least one of R_B' and R_B'' is OMe, OH or F.

[0128] In some embodiments, the nucleobase sequence of the oligonucleotide of Formulae (VIII) or (IX) comprises a sequence selected from those in Table A. In some embodiments, the nucleobase sequence of the oligonucleotide of Formulae (VIII) or (IX) comprises a sequence 1, 2, 3, 4, or 5 nucleobases different from a sequence selected from those in Table H.

Table H

Nucleobase Sequence (5'-3')	SEQ ID NO
5'-GCAGAGGTGAAGCGAAGUGC-3'	1
5'-GCAGAGGTGAAGCGAAGUGC-Chol-3'	2
5'-GCAGAGGTGAAGCGAAGUGC-GalNAc-3'	3
5'-GAUUAGGCAGAGGTGAAAAAG-3'	4
5'-GAUUAGGCAGAGGTGAAAAAG-Chol-3'	5
5'-GAUUAGGCAGAGGTGAAAAAG-GalNAc-3'	6
5'-GAUUAGGCAGAGGTGAAAAAG-3'	7
5'-GAUUAGGCAGAGGTGAAAAAG-Chol-3'	8
5'-GAUUAGGCAGAGGTGAAAAAG-GalNAc-3'	9
5'-GDAPUUDAPGGCAGAGGTGAAAAAG-3'	10
5'-GAUUAGGCAGAGGTGAADAPDAPDAPG-3'	11
5'-GAUUAGGCAGAGGTGDAPDAPDAPDAPDAPG-3'	12
5'-GDAPUUDAPGGCAGAGGTGAADAPDAPDAPG-3'	13

5'-GDAPUUDAPGGCAGAGGTGDAPDAPDAPDAPDAPG-3'	14
5'-GDAPUUDAPGGCAGAGGTGAAAAAG-3'	15
5'-GAUUAGGCAGAGGTGAADAPDAPDAPG-3'	16
5'-GAUUAGGCAGAGGTGDAPDAPDAPDAPDAPG-3'	17
5'-GDAPUUDAPGGCAGAGGTGAADAPDAPDAPG-3'	18
5'-GDAPUUDAPGGCAGAGGTGDAPDAPDAPDAPDAPG-3'	19
5'-GCAGAGGTGAAGCGADAPGUGC-3'	20
5'-GCAGAGGTGAAGCGDAPDAPGUGC-3'	21
5'-GCAGDAPGGTGAAGCGDAPDAPGUGC-3'	22
5'-GCDAPGDAPGGTGAAGCGDAPDAPGUGC-3'	23
5'-CGTGCAGAGGTGAAGC-3-NH_2-G-3'	24
5'-GCAGAGGTGAAGCGAA-3-NH_2-G-3	25
5'-CGACGTGCAGAGGTGAAG-3-NH_2-C-3'	26
5'-GCAGAGGTGAAGCGAAGTG-3-NH_2-C-3'	27
5'-GCAGAGGTGAAGC-3-NH_2-G-3'	28
5'-CGTGCAGAGGTGAAG-3-NH_2-C-3'	29
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	30
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	31
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	32
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	33
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	34
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	35
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	36
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	37
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	38
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	39
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	40
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	41
5'-GCAGAGGTGAAGCGAAGTG-3-NH_2-C-3'	42
5'-AAGAGAGGTG5meCG5meC5meC5meC5meCGUGG-3'	43
5'-GGUGAAG5meCGAAGTG5meCA5meCA5meCG-3'	44
5'-5meCGUG5meCAGAGGTGAAG5meCGAAG-3'	45
5'-AGAGGTGAAG5meCGAAGUG5meCA5meC-3'	46
5'-UGG5meCA5meCTAGTAAA5meCTGAG5meC5meC-3'	47
5'-5meCUAGGAGTT5meC5meCG5meCAGUAUGG-3'	48
5'-AGAGGTG5meCG5meC5meC5meC5meCGTGGU5meCG-3'	49
5'-GAGGUG5meCG5meC5meC5meC5meCGTGGU5meCGG-3'	50
5'-GAAAG5meC5meC5meCTA5meCGAA5meC5meCA5meCUG-3'	51
5'-GUU5meC5meCG5meCAGTATGGAU5meCGG5meC-3'	52
5'-U5meC5meCG5meCAGTATGGAT5meCGG5meCAG-3'	53
5'-A5meC5meCA5meCTGAA5meCAAATGG5meCA5meCU-3'	54
5'-UG5meCAGAGGTGAAG5meCGAAGUG-3'	55
5'-A5meCUGAA5meCAAATGG5meCA5meCUAGU-3'	56
5'-AGU5meC5meCA5meC5meCA5meCGAGT5meCUAGA5meC-3'	57
5'-5meCA5meCUGAA5meCAAATGG5meCA5meCUAG-3'	58
5'-5meCAGAGGTGAAG5meCGAAGUG5meCA-3'	59
5'-AAGAGAGGTG5meCG5meC5meC5meC5meCGUGG-GalNAc-3'	60
5'-GGUGAAG5meCGAAGTG5meCA5meCA5meCG-GalNAc-3'	61
5'-UGG5meCA5meCTAGTAAA5meCTGAG5meC5meC-GalNAc-3'	62

5'-5meCUAGGAGTT5meC5meCG5meCAGUAUGGGalNAc-3'	63
5'-AGAGGTG5meCG5meC5meC5meC5meCGTGGU5meCGGalNAc-3'	64
5'-U5meC5meCG5meCAGTATGGAT5meCGG5meCAG-GalNAc-3'	65
5'-UG5meCAGAGGTGAAG5meCGAAGUGGalNAc-3'	66
5'-AGU5meC5meCA5meC5meCA5meCGAGT5meCUAGA5meC-GalNAc-3'	67
5'-GCGGGTGAAGCGGUG-3-NH_2-C-3'	68
5'-GCGGGTGAAGCGGUG-3-NH_2-C-3'	69
5'-GCGGGTGAAGCGGUG-3-NH_2-C-3'	70
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	71
5'-GCAGAGGTGAAGCGAGTG-3NH_2-C-3'	72
5'-GCAGAGGTGAAGCGAAGTG-3NH_2-C-3'	73
5'-GCAGnspAGGTGAAGCGAAGUGC-3'	74
5'-GCAGAGGTGAAGCGAAGUGC-3'	75
5'-GCAGAGGTGAAGCGAAGUGC-3'	76
5-GCUCCAAATTCTTTAUAAGGG-GalNAc-3	77
5'-AAGAGAGGTG5meCG5meC5meC5meC5meCGUGG-3'	78
5'-GGUGAAG5meCGAAGTG5meCA5meCA5meCG-3'	79
5-5meCGUG5meCAGAGGTGAAG5meCGAAG-3'	80
5'-GUGAAG5meCGAAGTG5meCA5meCA5meCGG-3'	81
5'-AGAGGTGAAG5meCGAAGUG5meCA5meC-3'	82
5'-UGG5meCA5meCTAGTAAA5meCTGAG5meC5meC-3'	83
5'-5meCUAGGAGTT5meC5meCG5meCAGUAUGG-3'	84
5'-G5meCAGAGGTGAAG5meCGAAGUG5meC-3'	85
5'-AGAGGTG5meCG5meC5meC5meC5meCGTGGU5meCG-3'	86
5'-GAGGUG5meCG5meC5meC5meC5meCGTGGU5meCGG-3'	87
5'-GAAAG5meC5meC5meCTA5meCGAA5meC5meCA5meCUG-3'	88
5'-GUU5meC5meCG5meCAGTATGGAU5meCGG5meC-3'	89
5'-U5meC5meCG5meCAGTATGGAT5meCGG5meCAG-3'	90
5'-A5meC5meCA5meCTGAA5meCAAATGG5meCA5meCU-3'	91
5'-UG5meCAGAGGTGAAG5meCGAAGUG-3'	92
5'-A5meCUGAA5meCAAATGG5meCA5meCUAGU-3'	93
5'-AGU5meC5meCA5meC5meCA5meCGAGT5meCUAGA5meC-3'	94
5'-5meCA5meCUGAA5meCAAATGG5meCA5meCUAG-3'	95
5'-5meCAGAGGTGAAG5meCGAAGUG5meCA-3'	96
5'-AAGAGAGGTG5meCG5meC5meC5meC5meCGUGG3'	97
5'-AAGAGAGGTG5meCG5meC5meC5meC5meCGUGG-3'	98
5'-GGUGAAG5meCGAAGTG5meCA5meCA5meCG3'	99
5'-GGUGAAG5meCGAAGTG5meCA5meCA5meCG3'	100
5'-UGG5meCA5meCTAGTAAA5meCTGAG5meC5meC3'	101
5'-UGG5meCA5meCTAGTAAA5meCTGAG5meC5meC3'	102
5'-5meCUAGGAGTT5meC5meCG5meCAGUAUGG3'	103
5'-5meCUAGGAGTT5meC5meCG5meCAGUAUGG3'	104
5'-GCAGAGGTGAAGCGAAG-3'	105
5'-GCAGAGGTGAAGCGAAGTGC-3'	106
5'-CGTGCAGAGGTGAAGCG-3'	107
5'-GCAGAGGTGAAGCGAAG-3'	108
5'-CGACGTGCAGAGGTGAAGC-3'	109
5'-GCAGAGGTGAAGCGAAGTGC-3'	110
5'-GCAGAGGTGAAGCG-3'	111

5’-CGTGCAGAGGTGAAGC-3’	112
5’-GCAGAGGTGAAGCGAAGTG-3nh2-C-3’	113
5’-GalNAc-NHC6-U5meC5meCG5meCAGTATGGAT5meCGG5meCAG3’	114
5’-GalNAc-NHC6-5meCUAGGAGTT5meC5meCG5meCAGUAUGG3’	115
5’-GalNAc-NHC6-AAGAGAGGTG5meCG5meC5meC5meC5meCGUGG3’	116
5’GalNAc-NHC6-AGAGGTG5meCG5meC5meC5meC5meCGTGGU5meCG3’	117
5’GalNAc-NHC6-UG5meCAGAGGTGAAG5meCGAAGUG3’	118
mGCUCCAAATTCTTTAUAAGG	119
mGCUCCAAATTCTTTAUAAGG	120
mGCUCCAAATTCTTTAUAAGGG	121
mGCUCCAAATTCTTTAUAAGG/GalNAc/	122
mGCUCCAAATTCTTTAUAAGG/GalNAc/	123
mGCUCCAAATTCTTTAUAAGG/3CholTEG/	124
mGCUCCAAATTCTTTAUAAGG/3CholTEG/	125
mGCUCCAAATTCTTTAUAAGGG/3CholTEG/	126
5’-mG5mCAGAGGTGAAGp5mCGAAGUG5meC-3	127
5’-mG5mCAGAGGTGAAG5mCGAAGUG5mC-Cholesterol-3’	128
5’-mG5mCAGAGGTGAAGp5mCGAAGUG5mC-TEG-Cholesterol-3’	129
5’-mG5mCAGAGGTGAAG5mCGAAGUG5mC-Tocopherol-3’	130
5’-mG5mCAGAGGTGAAG5mCGAAGUG5mC-TEG-Tocopherol-3’	131
5’-mG5mCAGAGGTGAAG5mCGAAGUG5mC-GalNAc-3’	132
5’-mG5meCAGAGGTGAAG5meCGAAGUG5meC-3’	133
5’-mG5meCAGAGGTGAAG5meCGAAGUG5meC-po-Chol-3’	134
5’-mG5meCAGAGGTGAAG5meCGAAGUG5meC-po-Tocopherol-3’	135
5’-mG5meCAGAGGTGAAG5meCGAAGUG5meC-po-GalNAc-3’	136
5’-mG5meCAGAGGTGAAG5meCGAAGUG5meC-3’	137
5’-mG5meCAGAGGTGAAG5meCGAAGUG5meC-po-Chol-3’	138
5’-mG5meCAGAGGTGAAG5meCGAAGUG5meC-po-Tocopherol-3’	139
5’-mG5meCAGAGGTGAAG5meCGAAGUG5meC-po-GalNAc-3’	140
5-mG5meCAGAGGTGAAG5meCGAAGUG5meC-3	141
5-mG5meCAGAGGTGAAG5meCGAAGUG5meC-Chol-3	142
5-mG5meCAGAGGTGAAG5meCGAAGUG5meC-Toco-3	143
5-mG5meCAGAGGTGAAG5meCGAAGUG5meC-GalNAc-3	144
5-G5meCAGAGGTGAAG5meCGAAGUG5meC-3	145
5-G5meCAGAGGTGAAG5meCGAAGUG5meC-Chol-3	146
5-G5meCAGAGGTGAAG5meCGAAGUG5meC-Toco-3	147
5-G5meCAGAGGTGAAG5meCGAAGUG5meC-GalNAc-3	148
5-G5meCAGAGGTGAAG5meCGAAGUG5meC-3	149
5-dTGCAGAGGTGAAGCGAAGTG-3	150
5-dTGCAGAGGTGAAGCGAAGUG3’	151
5-GCAGAGGTGAAGCGAAGUGC-3’	152
5-GCAGAGGTGAAGCGAAGUGC-3’	153
5’-GCAGAGGTGAAGCGAAGUGC-3’	154
5’-dGCAGAGGTGAAGCGAAGUGC-3’	155
5’-dGCAGAGGTGAAGCGAAGUGC-3’	156
5’-dGCAGAGGTGAAGCGAAGUGC-3’	157
5’-dGCAGAGGTGAAGCGAAGUGC-3’	158

[0129] In embodiments, the disclosed oligonucleotides display an affinity for at least one of the six sequences of the HBV genome or its RNA equivalents and/or display stability complexed to at least one of the following six sequences of the HBV genome (Table E) or its RNA equivalents (Table F). In embodiments, the oligonucleotide complexed with a complementary HBV genome sequence has a melting temperature (Tm) of >37 °C. The HBV genome may be an RNA sequence such as DR-1 and/or DR-2 RNA sequence. The complex may be formed under physiological conditions or nearly physiological conditions such as in phosphate-buffered saline (PBS). In embodiments, the Tm of the complex is >50 °C. In embodiments, the Tm of the complex is 50-100 °C. In embodiments, the Tm of a disclosed oligonucleotide duplexed with an HBV RNA under physiological conditions or nearly physiological conditions is >50 °C.

[0130] In some aspects of the disclosure, the nucleobase sequence of the oligonucleotide of Formula (VIII) or (IX) comprises a sequence of 12-22 nucleotides, for example, 14-20 nucleotides or 16-19 nucleotides. In some embodiments, the nucleobase sequence of the oligonucleotide of Formula (VIII) or (IX) is 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 nucleotides in length.

[0131] In another aspect of the disclosure, the oligonucleotides described herein are conjugated or modified at one or more end.

[0132] For example, in some embodiments, a terminal end of the oligonucleotide is protected from hydrolytic cleavage by at least one modified nucleotide at said terminal end. In some embodiments, the modified nucleotide is a modified nucleotide comprising a modified nucleotide comprising a 3'-N modification, and may include a thiophosphoramidate subunit linkage. In some embodiments, the oligonucleotides of Formulae (VIII) and (IX) further comprise at least one nucleotide (e.g. 1 or 2) at the 3' and/or 5' end that contains a thiophosphate intersubunit linkage and a thymine nucleobase. In some embodiments, the oligonucleotides of Formulae (VIII) and (IX) further comprise at least one nucleotide (e.g. 1 or 2) at the 3' and/or 5' end that contains a 2'-OMe modified nucleotide and a thymine nucleobase. In some embodiments, the oligonucleotides of Formulae (VIII) and (IX) further comprise at least one 2'-OMe modified nucleotide at the 3' and/or 5' end that contains a thiophosphate intersubunit linkage and a uracil nucleobase. In some embodiments, the an inverted dT can be incorporated at the 3'-end of the

oligonucleotides of Formulae (VIII) and (IX), leading to a 3'-3' linkage which may inhibit degradation by 3' exonucleases and/or extension by DNA polymerases.

D. Conjugated Oligonucleotides

[0133] The present disclosure is also directed to additional components conjugated to the oligonucleotide such as targeting moieties and oligonucleotides modified at one or more ends.

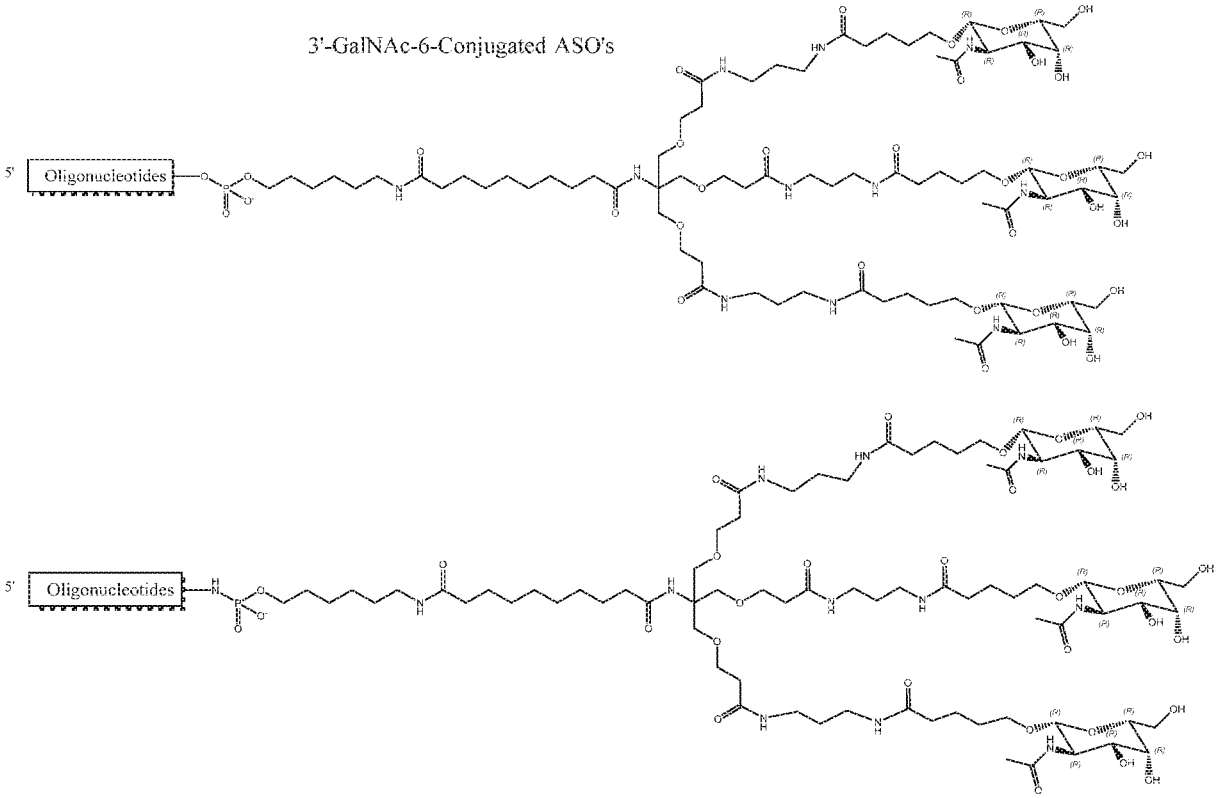
[0134] In some embodiments, the oligonucleotides described herein are conjugated to one or more ligand targeting group or pharmacophore, optionally through a linking moiety, such as a HEG linker or a C6 or C7 amino linker. In some embodiments, oligonucleotides described herein further comprises a ligand targeting group or a pharmacophore conjugated at the 5' and/or 3' end through an optional linker. In preferred embodiments, the oligonucleotides described herein further comprise a ligand-targeting group conjugated at the 5' and/or 3' end through an optional linker. In some embodiments, the conjugation is at the 3'-end of the oligonucleotides described herein.

[0135] In some embodiments, the ligand-targeting group or a pharmacophore enhances the activity, cellular distribution or cellular uptake of the oligonucleotide by a particular type of cell such as hepatocytes.

[0136] In some embodiments, the ligand targeting group may be a lipid moiety such as a cholesterol moiety, tocopherols, cholic acid, a thioether, e.g., beryl-S-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid, a palmitoyl moiety, or an octadecylamine or hexylaminocarbonyloxycholesterol moiety

[0137] For example, in some embodiments, a terminal end of the oligonucleotide is protected from hydrolytic cleavage by at least one modified nucleotide at the terminal end. In some embodiments, the modified nucleotide is a modified nucleotide comprising a modified nucleotide comprising a 3'-N modification, and may include a thiophosphoramidate subunit linkage. In some embodiments, the oligonucleotide strand further comprises at least one nucleotide (e.g. 1 or 2) at the 3' and/or 5' end that contains a thiophosphate intersubunit linkage and a thymine nucleobase. In some embodiments, the oligonucleotide strand further comprises at least one

nucleotide (e.g. 1 or 2) at the 3' and/or 5' end that contains a 2'-F, 2'-OMe, 2'-OEt, or 2'-MOE modified nucleotide. In some embodiments, the oligonucleotide strand further comprises at least one 2'-OMe modified nucleotide at the 3' and/or 5' end that contains a thiophosphate intersubunit linkage and a uracil nucleobase. In embodiments, the 3' end of the ASO is attached through an np or po linkage to a C6 amino linker further linked to GalNAc-6. For example, the following structures can exemplify this construct:



In some embodiments, an inverted dT can be incorporated at the 3'-end of the oligonucleotide strand, leading to a 3'-3' linkage that may inhibit degradation by 3' exonucleases and/or extension by DNA polymerases.

[0138] In some embodiments, the oligonucleotides described herein are conjugated to one or more ligand targeting group or pharmacophore, optionally through a linking moiety, such as a HEG linker or a C6 amino linker. In some embodiments, the oligonucleotide strand further comprises a ligand-targeting group or a pharmacophore conjugated at the 5' and/or 3' end through an optional linker. In some embodiments, the conjugation is at the 3'-end of the oligonucleotide strand.

[0139] In some embodiments, the ligand-targeting group or a pharmacophore enhances the activity, cellular distribution, or cellular uptake of the oligonucleotide by a particular type of cell such as hepatocytes.

[0140] In some embodiments, the ligand targeting group may be a lipid moiety such as a cholesterol moiety, tocopherols, cholic acid, a thioether, e.g., beryl-S-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid, a palmitoyl moiety, or an octadecylamine or hexylaminocarbonyloxycholesterol moiety.

[0141] In some embodiments, the ligand-targeting group may be a naturally occurring substance, such as a protein (e.g., human serum albumin (HSA), low-density lipoprotein (LDL), or globulin).

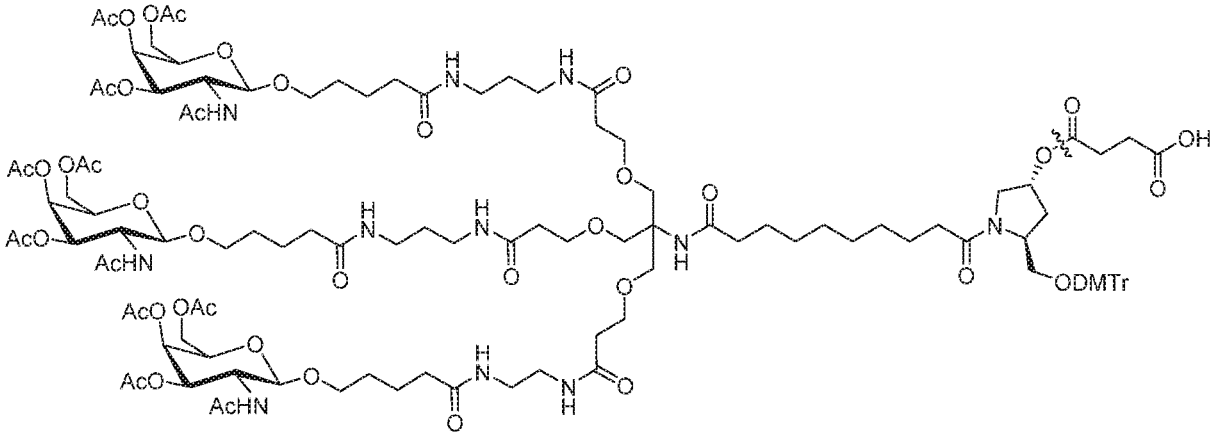
[0142] In some embodiments, the ligand-targeting group may be a carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin, N-acetylgalactosamine, or hyaluronic acid). Carbohydrates include monosaccharides such as N-acetylgalactosamine (GalNAc), disaccharides, trisaccharides, tetrasaccharides, oligosaccharides, and polysaccharides. In certain embodiments of the compositions and methods of the invention, a ligand is one or more GalNAc derivatives attached such as two or three GalNAc derivatives attached to the oligonucleotide through a bivalent or trivalent-branched linker, respectively.

[0143] In embodiments, the oligonucleotide is linked to the targeting moiety through a linker, such as an amino alkyl linker (e.g., C6-NH_2). For example, GAlNAc -1-6 may be linked to the oligonucleotide through this type of linker.

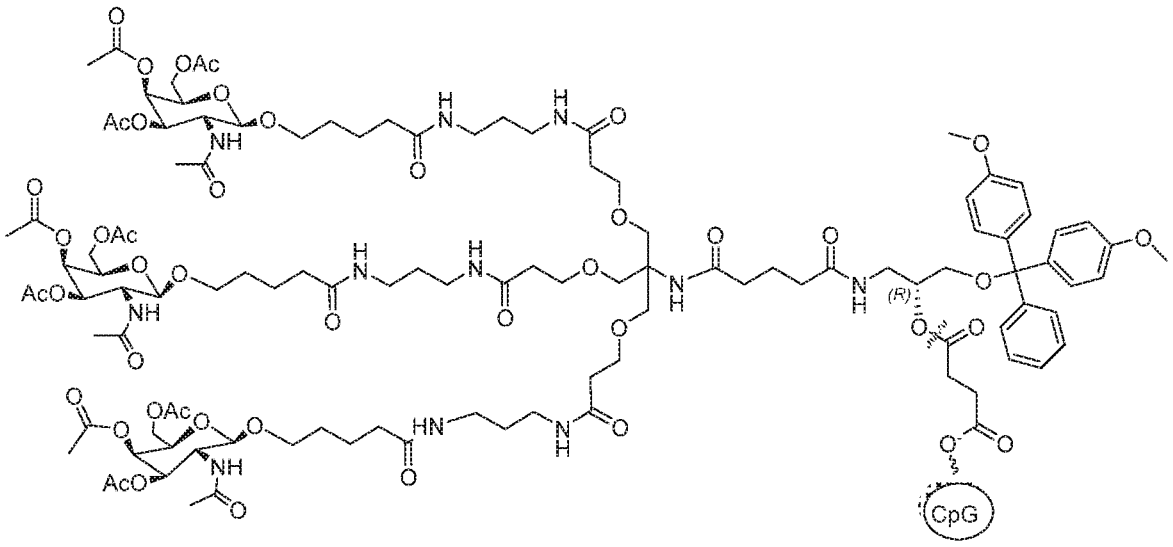
[0144] In some embodiments, the ligand-targeting group may be a recombinant or synthetic molecule, such as a synthetic polymer, e.g., a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-

polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide. The ligand targeting group can also include targeting groups, e.g., a cell or tissue targeting agent, e.g., a lectin, glycoprotein, lipid or protein, e.g., an antibody, that binds to a specified cell type such as an hepatocyte.

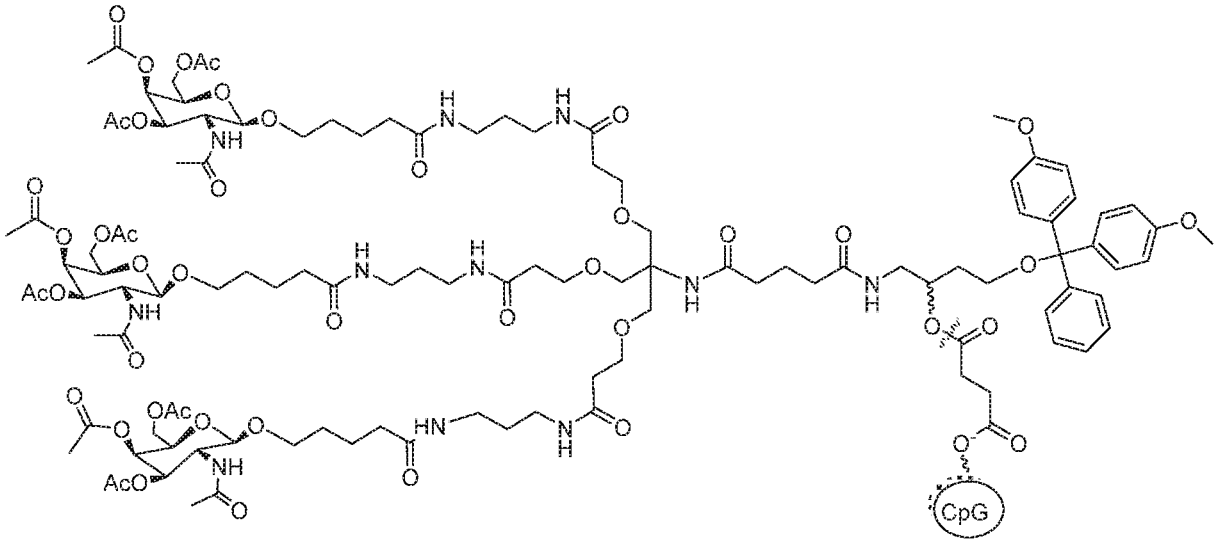
[0145] In some embodiments, the ligand-targeting group is GalNAc or a derivative thereof. For example, the following GalNAc derivatives are included in some embodiments.



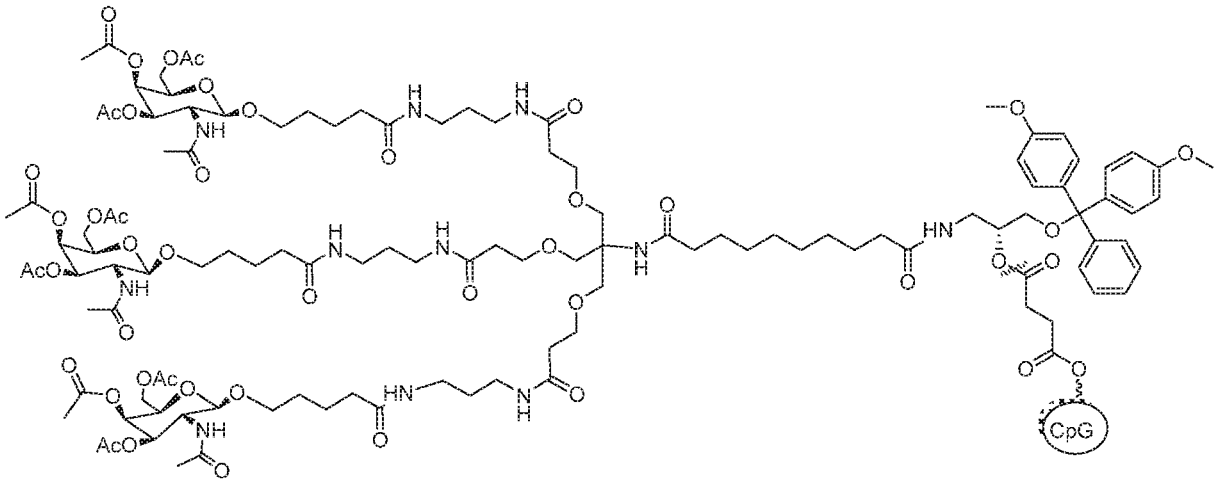
GalNAc-1



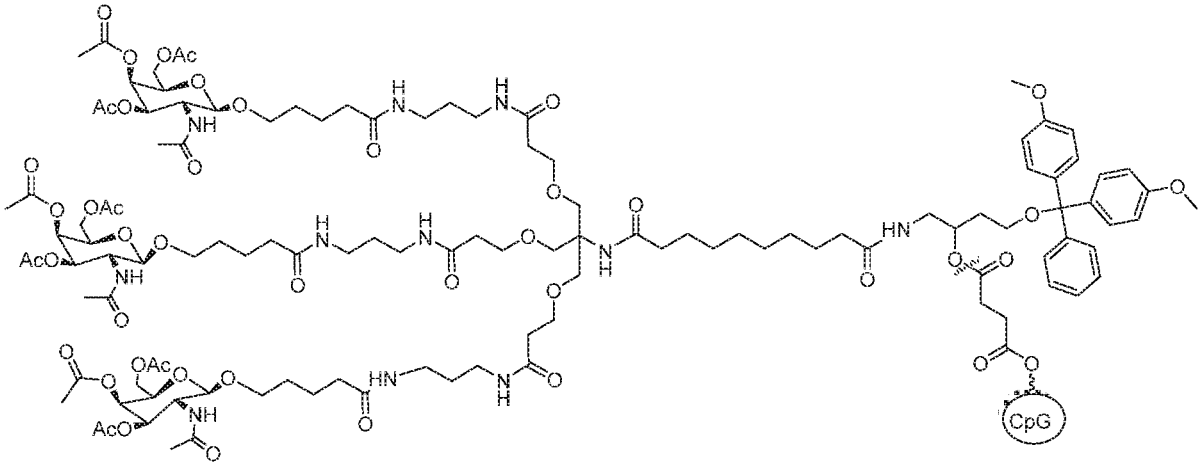
GalNAc-2-CPG



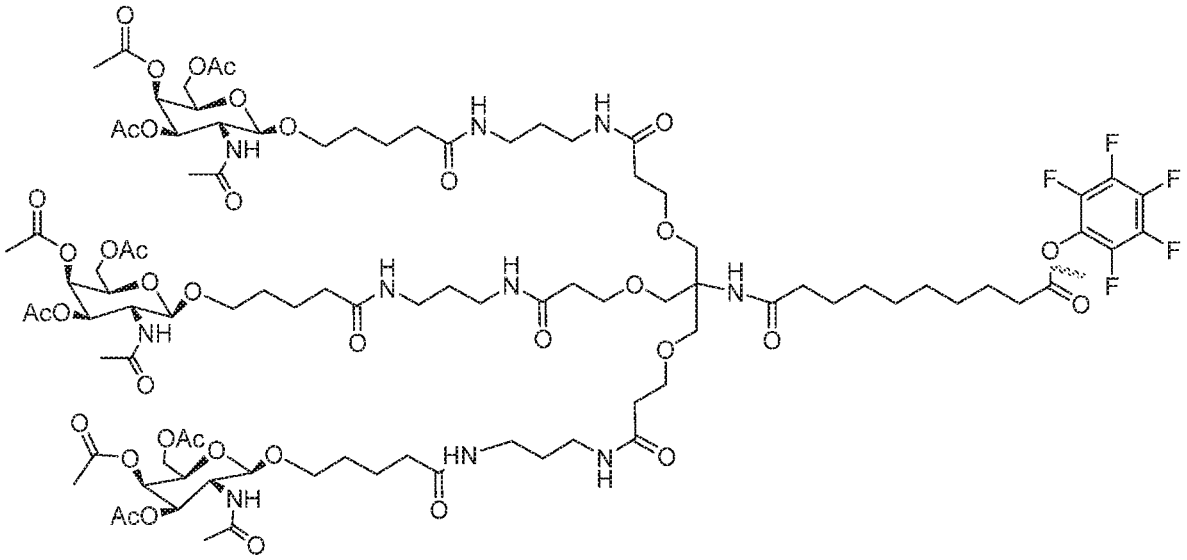
GalNAc-3



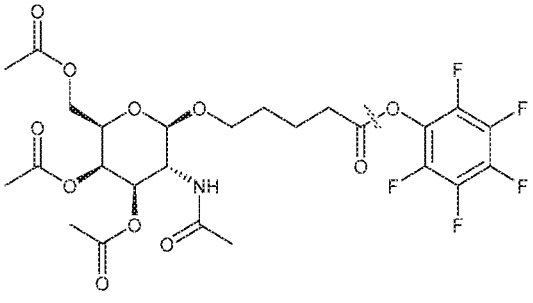
GalNAc-4



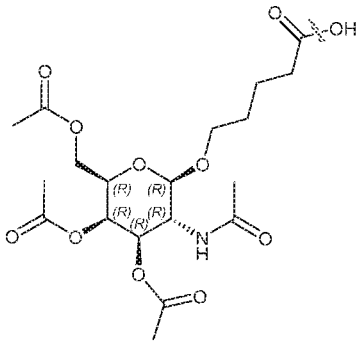
GalNAc-5



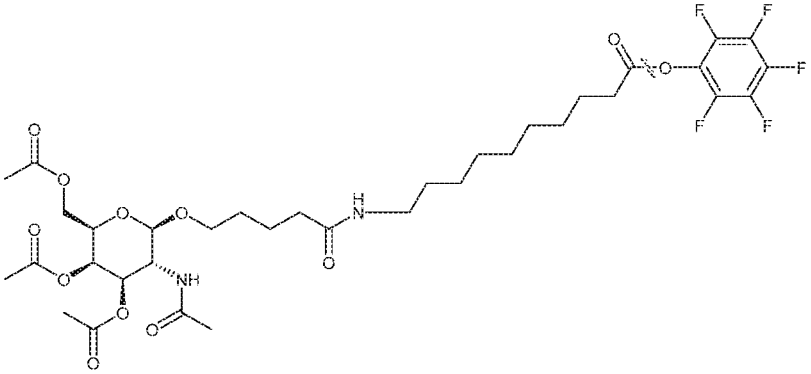
GalNAc-6



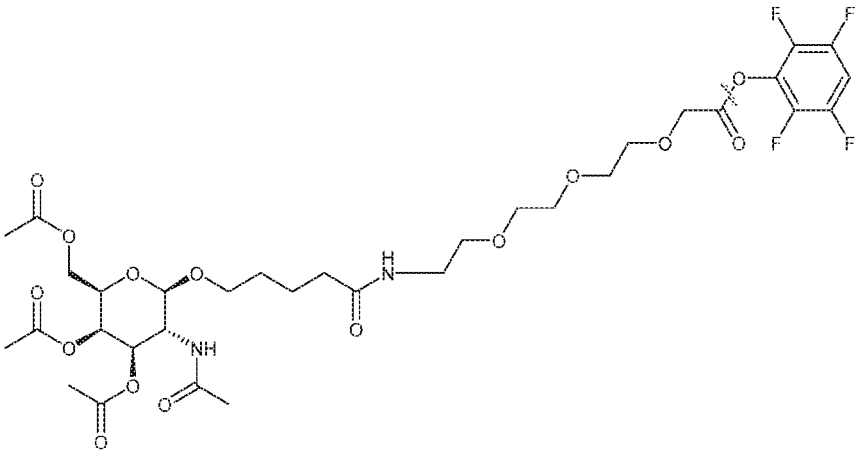
GalNAc-7



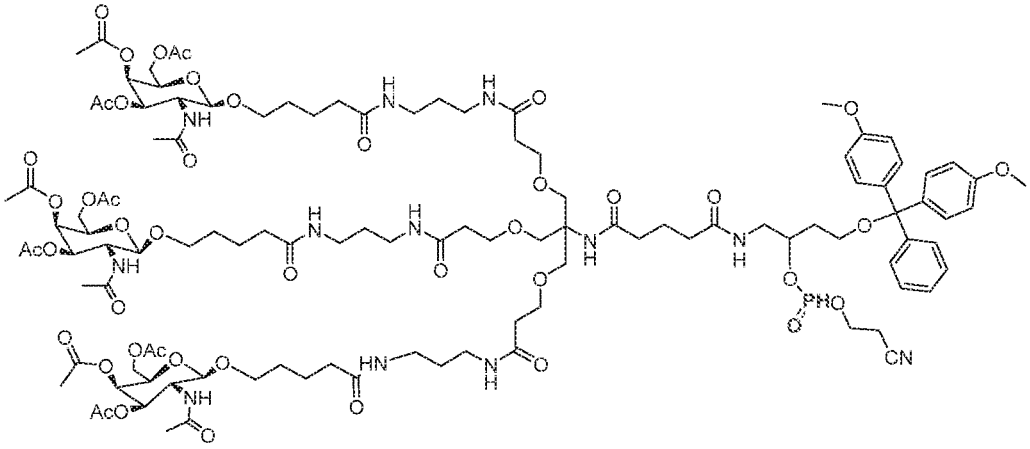
GalNAc-8



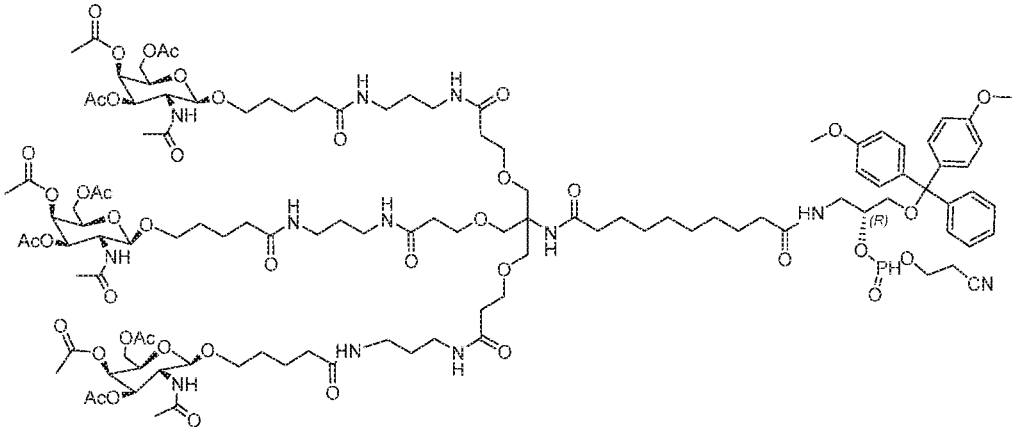
GalNAc-9



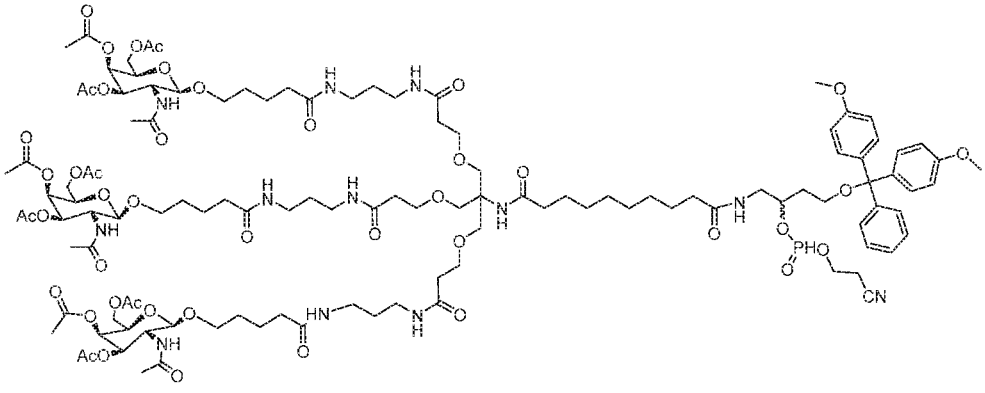
GalNAc-10



GalNAc-11



GalNAc-12



GalNAc-13

[0146] In some embodiments, the ligand-targeting group may be an aptamer. An "aptamer" refers to an oligonucleotide or peptide molecule that binds to a specific target molecule. For

example, an aptamer can be selected to target a specific cell type in the body. When conjugated to the disclosed oligonucleotide, it can direct the oligonucleotide towards the targeted cells. In another example, an aptamer may target a viral protein, such as the core protein of HBV. See, e.g., Oncogene, 2001 Oct 4;20(45):6579-86; WO2011060557. The aptamer may specifically bind the reverse transcriptase primer or HBV reverse transcriptase or HBV Enhancer I core sequence, for example, as described in WO2002081494.

[0147] In some embodiments, the ligand targeting group may be selected from one or more of a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucoseamine multivalent mannose, multivalent fructose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B 12, vitamin A, biotin, a RGD peptide, or a RGD peptide mimetic.

[0148] Additional ligand targeting groups are disclosed, e.g., in WO2016077321, which is incorporated herein by reference in its entirety.

2. Compositions

[0149] The present disclosure also encompasses pharmaceutical compositions comprising oligonucleotides of the present disclosure. One embodiment is a pharmaceutical composition comprising an oligonucleotide of Formula (I), (II), (III), (IV), (V), or (VI), or other oligonucleotide of the present disclosure and a pharmaceutically acceptable diluent or carrier.

[0150] In some embodiments, the pharmaceutical composition containing the oligonucleotide of the present disclosure is formulated for systemic administration via parenteral delivery. Parenteral administration includes intravenous, intra-arterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; also subdermal administration, e.g., via an implanted device. In a preferred embodiment, the pharmaceutical composition containing the oligonucleotide of the present disclosure is formulated for subcutaneous (SC) or intravenous (IV) delivery. Formulations for parenteral administration may include sterile aqueous solutions, which may also contain buffers, diluents and other pharmaceutically acceptable additives as understood by the skilled artisan. For intravenous use, the total concentration of solutes may be controlled to render the preparation isotonic.

[0151] The pharmaceutical compositions containing the oligonucleotide of the present disclosure are useful for treating a disease or disorder, e.g., associated with the expression or activity of an HBV gene.

3. Methods of Use

[0152] One aspect of the present technology includes methods for treating a subject diagnosed as having, suspected as having, or at risk of having an HBV infection and/or an HBV-associated disorder. In therapeutic applications, compositions comprising the oligonucleotides of the present technology are administered to a subject suspected of, or already suffering from such a disease (such as, *e.g.*, presence of an such as HBV antigen surface and envelope antigens (*e.g.*, HBsAg and/or HBeAg) in the serum and/or liver of the subject, or elevated HBV DNA or HBV viral load levels), in an amount sufficient to cure, or at least partially arrest, the symptoms of the disease, including its complications and intermediate pathological phenotypes in development of the disease.

[0153] In some embodiments the oligonucleotides of the present technology show affinity to at least one of the following regions or HBV RNA transcripts in Table J.

Table J

Region	Targeted HBV RNA transcripts	HBV Proteins affected
Pol/S	Pre-Core, Pg, Pre-S1, Pre-S2	HBeAg, HBcAg, Polymerase, Large HBsAg, Middle HBsAg, Small HBsAg
Pol	Pre-Core, Pg, Pre-S1, Pre-S2	HBeAg, HBcAg, Polymerase, Large HBsAg, Middle HBsAg, Small HBsAg
Pol/X	Pre-Core, Pg, Pre-S1, Pre-S2, X	HBeAg, HBcAg, Polymerase, Large HBsAg, Middle HBsAg, Small HBsAg, HBxAg
DR1	Pre-Core, Pg, Pre-S1, Pre-S2, X	HBeAg, HBcAg, Polymerase, Large HBsAg, Middle HBsAg, Small HBsAg, HBxAg
DR2	Pre-Core, Pg, Pre-S1, Pre-S2, X	HBeAg, HBcAg, Polymerase, Large HBsAg, Middle HBsAg, Small HBsAg, HBxAg
Pre-PolyA	Pre-Core, Pg, Pre-S1, Pre-S2, X	HBeAg, HBcAg, Polymerase, Large HBsAg, Middle HBsAg, Small HBsAg, HBxAg

[0154] Subjects suffering from an HBV infection and/or an HBV-associated disorder can be identified by any or a combination of diagnostic or prognostic assays known in the art. For example, typical symptoms of HBV infection and/or an HBV-associated disorder include, but are not limited to the presence of serum and/or liver HBV antigen (*e.g.*, HBsAg and/or HBeAg), elevated ALT, elevated AST, the absence or low level of anti-HBV antibodies, liver injury, cirrhosis, delta hepatitis, acute hepatitis B, acute fulminant hepatitis B, chronic hepatitis B, liver fibrosis, end-stage liver disease, hepatocellular carcinoma, serum sickness-like syndrome, anorexia, nausea, vomiting, low-grade fever, myalgia, fatigability, disordered gustatory acuity and smell sensations (aversion to food and cigarettes), right upper quadrant and epigastric pain (intermittent, mild to moderate), hepatic encephalopathy, somnolence, disturbances in sleep pattern, mental confusion, coma, ascites, gastrointestinal bleeding, coagulopathy, jaundice, hepatomegaly (mildly enlarged, soft liver), splenomegaly, palmar erythema, spider nevi, muscle wasting, spider angiomas, vasculitis, variceal bleeding, peripheral edema, gynecomastia, testicular atrophy, abdominal collateral veins (caput medusa), high levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (within a range of 1000-2000 IU/mL), ALT levels higher than AST levels, elevated gamma-glutamyl transpeptidase (GGT) and/or alkaline phosphatase (ALP) levels, decreased albumin levels, elevated serum iron levels, leukopenia (*i.e.*, granulocytopenia), lymphocytosis, increased erythrocyte sedimentation rate (ESR), shortened red blood cell survival, hemolysis, thrombocytopenia, a prolongation of the international normalized ratio (INR), the presence of serum HBV DNA, elevation of the aminotransferases (<5 times the ULN), increased bilirubin levels, prolonged prothrombin time (PT), hyperglobulinemia, the presence of tissue-nonspecific antibodies, such as anti-smooth muscle antibodies (ASMAs) or antinuclear antibodies (ANAs), the presence of tissue-specific antibodies, such as antibodies against the thyroid gland, elevated levels of rheumatoid factor (RF), hyperbilirubinemia, low platelet and white blood cell counts, AST levels higher than ALT levels, lobular inflammation accompanied by degenerative and regenerative hepatocellular changes, and predominantly centrilobular necrosis.

[0155] In some embodiments, subjects treated with the oligonucleotide composition of the present technology will show amelioration or elimination of one or more of the following conditions or symptoms: the presence of serum and/or liver HBV antigen (*e.g.*, HBsAg and/or HBeAg), the absence or low level of anti-HBV antibodies, liver injury, cirrhosis, delta hepatitis,

acute hepatitis B, acute fulminant hepatitis B, chronic hepatitis B, liver fibrosis, end-stage liver disease, hepatocellular carcinoma, serum sickness-like syndrome, anorexia, nausea, vomiting, low-grade fever, myalgia, fatigability, disordered gustatory acuity and smell sensations (aversion to food and cigarettes), right upper quadrant and epigastric pain (intermittent, mild to moderate), hepatic encephalopathy, somnolence, disturbances in sleep pattern, mental confusion, coma, ascites, gastrointestinal bleeding, coagulopathy, jaundice, hepatomegaly (mildly enlarged, soft liver), splenomegaly, palmar erythema, spider nevi, muscle wasting, spider angiomas, vasculitis, variceal bleeding, peripheral edema, gynecomastia, testicular atrophy, abdominal collateral veins (caput medusa), ALT levels higher than AST levels, leukopenia (*i.e.*, granulocytopenia), decreased albumin levels, elevated serum iron levels, lymphocytosis, increased erythrocyte sedimentation rate (ESR), shortened red blood cell survival, hemolysis, thrombocytopenia, a prolongation of the international normalized ratio (INR), the presence of serum HBV DNA, prolonged prothrombin time (PT), hyperglobulinemia, the presence of tissue-nonspecific antibodies, such as anti-smooth muscle antibodies (ASMAs) or antinuclear antibodies (ANAs), the presence of tissue-specific antibodies, such as antibodies against the thyroid gland, hyperbilirubinemia, low platelet and white blood cell counts, AST levels higher than ALT levels, lobular inflammation accompanied by degenerative and regenerative hepatocellular changes, and predominantly centrilobular necrosis.

[0156] In some embodiments, subjects treated with the oligonucleotide composition of the present technology will show a reduction in the expression levels of one or more biomarkers selected from among alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), bilirubin, and rheumatoid factor (RF), compared to untreated subjects suffering from an HBV infection and/or an HBV-associated disorder.

[0157] The present disclosure provides a method for treating a subject diagnosed as having, or suspected as having an HBV infection and/or an HBV-associated disorder comprising administering to the subject an effective amount of an oligonucleotide composition of the present technology.

[0158] The oligonucleotides and compositions of the present disclosure may be used in antisense therapy. For example, the oligonucleotide may contain a nucleobase sequence that is

complementary or hybridizes to a target nucleic acid sequence of a known viral DNA or RNA sequence, for example, in HBV.

[0159] Some embodiments include a method of modulating expression of a target by contacting a target nucleic acid with an antisense compound comprising the oligonucleotide of the present disclosure. In some embodiments, the target nucleic acid is in a cell, for example, in an animal such as a human.

[0160] Some embodiments, include a method of inhibiting expression of a target RNA in an animal, comprising administering to the animal an antisense compound comprising the oligonucleotide of the present disclosure. The oligonucleotide may be complementary or hybridize to a portion of the target RNA.

[0161] Some embodiments include a method for reducing the viral load of a virus in a subject infected with the virus comprising administering a therapeutically effective amount of a oligonucleotide or a composition of the present disclosure to the subject in need thereof thereby reducing the viral load of the virus in the subject. The oligonucleotide may be complementary or hybridize to a portion of the target RNA in the virus.

[0162] Some embodiments include a method for inhibition of viral gene expression in a cell or subject comprising contacting the cell with a oligonucleotide or a composition of the present disclosure, or administering a therapeutically effective amount of a oligonucleotide or a composition of the present disclosure to a subject in need thereof. The oligonucleotide may be complementary or hybridize to a portion of the target RNA in the virus.

[0163] Other embodiments include a method of reducing the level of a virus antigen in a subject infected with the virus, comprising administering a therapeutically effective amount of a oligonucleotide or composition of the present disclosure to the subject in need thereof thereby reducing the level of the virus antigen in the subject. The oligonucleotide may be complementary or hybridize to a portion of the target RNA in the virus.

[0164] The oligonucleotides and compositions of the present disclosure may be used, e.g., to inhibit or reduce Hepatitis B virus (HBV) gene expression or inhibit replication of a HBV virus or for treatment of a subject having HBV or for reducing the viral load of Hepatitis B virus

(HBV) in a subject infected with HBV. In embodiments, the disclosed chimeric oligonucleotides are used to induce RNase H activity at a target gene.

[0165] The oligonucleotides and compositions of the present disclosure may be used, e.g., to compete for a micro-RNA binding site to HCV RNA thereby inhibiting replication.

[0166] The present disclosure is also directed to methods of stabilizing an oligonucleotide for delivery to a subject. Stabilization of an oligonucleotide is characterized [quantified] herein as increasing the melting point or temperature, T_m, of an oligonucleotide.

[0167] The disclosed oligonucleotide constructs may be administered alone or in combination with one or more additional treatments for the targeted ailment. The disclosed oligonucleotide constructs may be administered alone or in combination with one or more additional treatments for HBV infection. In combination therapies, it is understood that the oligonucleotide constructs and one or more additional treatments for HBV infection may be administered simultaneously in the same or separate compositions, or administered separately, at the same time or sequentially.

[0168] In some embodiments, the disclosed oligonucleotide constructs are administered in combination with HBV replication inhibitors or immune modulator agents or in regimens that combine anti-HBV oligonucleotide agents with both HBV replication inhibitors and immune modulation agents. In embodiments, the disclosed oligonucleotide constructs are administered in combination with standard of care treatment for HBV infection. Standard of care treatment for HBV infection can include inhibitors of viral polymerase such as nucleotide/nucleotide analogs (e.g., Lamivudine, Telbivudine, Entecavir, Adefovir, Tenofovir, and Clevudine, Tenofovir alafenamide (TAF), CMX157, and AGX-1009) and Interferons (e.g., Peg-IFN-2a and IFN-a-2b, Interferon lambda). In embodiments, the disclosed oligonucleotide constructs are administered in combination with one or more oligonucleotides after either simultaneous (co-administration) or sequential dosing. Oligonucleotides can include siRNA such as ALN-HBV, ARB-1467, ARC-520 and ARC-521, antisense oligonucleotides such as RG6004 (LNA HBV), Ionis-HBV$_{Rx}$ and Ionis-HBV-L$_{Rx}$, miRNA mimics or inhibitors, aptamers, steric blockers, saRNA, shRNA, immunomodulatory and/or HBsAg release inhibiting such as REP 2139 and REP 2165 oligonucleotides. In embodiments, the disclosed oligonucleotide constructs are administered in combination with one or more antiviral agents such as viral replication inhibitors. In embodiments, the disclosed oligonucleotide constructs are administered in combination with

HBV Capsid inhibitors. HBV capsid inhibitors can include NVR 3-778, AB-423, GLS-4, Bayer 41-4109, HAP-1, and AT-1. In embodiments, the disclosed oligonucleotide constructs are administered in combination with one or more immunomodulators such as TLR agonists. TLR agonists can include GS-9620, ARB-1598, ANA975, RG7795(ANA773), MEDI9197, PF-3512676, and IMO-2055. In embodiments, the disclosed oligonucleotide constructs are administered in combination with HBV vaccines. HBV vaccines can include Heplislav, ABX203, and INO-1800. In embodiments, the disclosed oligonucleotide constructs are administered in combination

[0169] Some embodiments include inhibition of HBV gene expression in a cell or subject comprising contacting the cell with an oligonucleotide or composition of the present disclosure, or administering a therapeutically effective amount of a oligonucleotide or composition of the present disclosure to a subject in need thereof.

[0170] Some embodiments include the treatment of a disease or disorder associated with the expression or activity of a HBV gene comprising administering a therapeutically effective amount of an oligonucleotide or composition of the present disclosure to a subject in need thereof.

[0171] Some embodiments include a method for reducing the viral load of Hepatitis B virus (HBV) in a subject infected with HBV comprising administering a therapeutically effective amount of an oligonucleotide or composition of the present disclosure to the subject in need thereof thereby reducing the viral load of HBV in the subject. Some embodiments also provide methods of reducing the viral load of Hepatitis D virus (HDV) in a subject infected with HDV.

[0172] Other embodiments include a method of reducing the level of a Hepatitis B virus (HBV) antigen in a subject infected with HBV comprising administering a therapeutically effective amount of an oligonucleotide or composition of the present disclosure to the subject in need thereof thereby reducing the level of the HBV antigen in the subject. Some embodiments also provide methods of reducing the level of a Hepatitis D virus (HDV) antigen in a subject infected with HDV. In some embodiments, the HBV antigen is HBsAg or HBeAg.

[0173] In one embodiment, an oligonucleotide or composition of the present disclosure targeting HBV is administered to a subject having an HBV infection or both and HBV and an HDV infection, and/or an HBV-associated disease such that the expression of one or more HBV genes,

HBV ccc DNA levels, HBV antigen levels, HBV viral load levels, ALT, and/or AST, e.g., in a cell, tissue, blood or other tissue or fluid of the subject are reduced by at least about 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41 %, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 62%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more, or values between two of these numbers, upon administration to the subject of the oligonucleotide or composition of the present disclosure. In some embodiments, the HBV antigen levels are decreased by the previously recited amount. In some embodiments the antigen is HBsAg or HBeAg. In some embodiments, the HBV viral load levels are decreased by the previously recited amount.

[0174] In one embodiment, a oligonucleotide or composition of the present disclosure targeting HBV is administered to a subject having an HBV infection or both and HBV and an HDV infection, and/or an HBV-associated disease such that the level of anti-HBV antibodies, e.g., in a cell, tissue, blood or other tissue or fluid of the subject are increased by at least about 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41 %, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 62%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more, or values between two of these numbers, when the an oligonucleotide or composition of the present disclosure is administered to the subject.

[0175] Administration of the oligonucleotide or composition of the present disclosure according to the methods and uses of the disclosure may result in a reduction of the severity, signs, symptoms, and/or markers of such diseases or disorders in a patient with an HBV infection or both and HBV and an HDV infection, and/or HBV-associated disease. By "reduction" in this context is meant a statistically significant decrease in such level. The reduction can be, for example, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or about 100%, or values between two of these numbers.

[0176] The amount of an oligonucleotide or composition of the present disclosure may be determined by a medical professional. The daily dosage of the products may be varied over a wide range from 0.001 to 1,000 mg per adult human per day, or any range therein. For oral administration, the compositions are preferably provided in the form of tablets containing, 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 150, 200, 250, and 500 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. An effective amount of the drug is ordinarily supplied at a dosage level of from about 0.01 mg/kg to about 100 mg/kg of body weight per day, or any range therein. Preferably, the range is from about 0.01 to about 50.0 mg/kg of body weight per day, or any range therein. More preferably, from about 0.01 to about 10.0 mg/kg of body weight per day, or any range therein. More preferably, from about 0.01 to about 1.0 mg/kg of body weight per day, or any range therein. The oligonucleotides may be administered on a regimen of 1 to 4 times per day. For example, the oligonucleotides of the present disclosure may be administered at one or more doses of from about 0.1 mg/kg to about 100 mg/kg. For example, the disclosed oligonucleotides may be administered at a dose of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 31, 32, 33, 34, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or about 100 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this disclosure. These values may apply to intravenous infusion and/or subcutaneous delivery. Other forms of delivery described herein may also be administered at these doses. The dosages may be varied depending upon the requirement of the patients, the severity of the condition being treated and the oligonucleotides being employed. The use of either daily administration or post-periodic dosing may be employed.

[0177] The oligonucleotides of the present disclosure can be administered by intravenous infusion over a period of time, such as over a 5, 6, 7, 8, 9, 10, 11 , 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or about a 25 minute period. The administration may be repeated, for

example, on a regular basis, such as weekly, biweekly (i.e., every two weeks) for one month, two months, three months, four months, or longer. After an initial treatment regimen, the treatments can be administered on a less frequent basis. For example, after administration weekly or biweekly for three months, administration can be repeated once per month, for six months or a year or longer.

[0178] The oligonucleotides of the present disclosure also can be administered by subcutaneous delivery. The administration may be repeated, for example, on a regular basis, such as weekly, biweekly (i.e., every two weeks) for one month, two months, three months, four months, or longer. After an initial treatment regimen, the treatments can be administered on a less frequent basis. For example, after administration weekly or biweekly for three months, administration can be repeated once per month, for six months or a year or longer.

[0179] Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of CHB may be assessed, for example, by periodic monitoring of viral load and transaminase levels. Comparison of the later readings with the initial readings provides an indication of whether the treatment is effective.

4. Definitions

[0180] It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present invention. The following definitions shall apply unless otherwise indicated.

[0181] The terms "complementary" or "complementarity" as used herein with reference to polynucleotides (*i.e.*, a sequence of nucleotides such as an oligonucleotide or a target nucleic acid) refer to the base-pairing rules. The complement of a nucleic acid sequence as used herein refers to an oligonucleotide which, when aligned with the nucleic acid sequence such that the 5' end of one sequence is paired with the 3' end of the other, is in "antiparallel association." For example, the sequence "5'-A-G-T-3'" is complementary to the sequence "3'-T-C-A-5." Certain

bases not commonly found in naturally occurring nucleic acids may be included in the nucleic acids described herein. These include, for example, inosine, 7-deazaguanine, Locked Nucleic Acids (LNA), and Peptide Nucleic Acids (PNA). Complementarity need not be perfect; stable duplexes may contain mismatched base pairs, degenerative, or unmatched bases. Those skilled in the art of nucleic acid technology can determine duplex stability empirically considering a number of variables including, for example, the length of the oligonucleotide, base composition, and sequence of the oligonucleotide, ionic strength, and incidence of mismatched base pairs. A complement sequence can also be an RNA sequence complementary to the DNA sequence or its complement sequence, and can also be a cDNA.

[0182] The term "hybridize" as used herein refers to a process where two substantially complementary nucleic acid strands (at least about 65% complementary over a stretch of at least 14 to 25 nucleotides, at least about 75%, or at least about 90% complementary) anneal to each other under appropriately stringent conditions to form a duplex or heteroduplex through formation of hydrogen bonds between complementary base pairs. Hybridizations are typically, and preferably, conducted with probe-length nucleic acid molecules, preferably 15-100 nucleotides in length, more preferably 18-50 nucleotides in length. Nucleic acid hybridization techniques are well known in the art. *See, e.g.*, Sambrook, *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Press, Plainview, N.Y. Hybridization and the strength of hybridization (*i.e.*, the strength of the association between the nucleic acids) is influenced by such factors as the degree of complementarity between the nucleic acids, stringency of the conditions involved, and the thermal melting point (T_m) of the formed hybrid. Those skilled in the art understand how to estimate and adjust the stringency of hybridization conditions such that sequences having at least a desired level of complementarity will stably hybridize, while those having lower complementarity will not. For examples of hybridization conditions and parameters, *see, e.g.*, Sambrook, *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Press, Plainview, N.Y.; Ausubel, F. M. *et al.* 1994, *Current Protocols in Molecular Biology*, John Wiley & Sons, Secaucus, N.J. In some embodiments, specific hybridization occurs under stringent hybridization conditions. An oligonucleotide or polynucleotide (*e.g.*, a probe or a primer) that is specific for a target nucleic acid will "hybridize" to the target nucleic acid under suitable conditions.

[0183] The term "stringent hybridization conditions" as used herein refers to hybridization conditions at least as stringent as the following: hybridization in 50% formamide, 5xSSC, 50 mM NaH_2PO_4, pH 6.8, 0.5% SDS, 0.1 mg/mL sonicated salmon sperm DNA, and 5x Denhart's solution at 42° C overnight; washing with 2x SSC, 0.1% SDS at 45° C; and washing with 0.2x SSC, 0.1% SDS at 45° C. In another example, stringent hybridization conditions should not allow for hybridization of two nucleic acids, which differ over a stretch of 20 contiguous nucleotides by more than two bases.

[0184] The term "substantially complementary" as used herein means that two sequences hybridize under stringent hybridization conditions. The skilled artisan will understand that substantially complementary sequences need not hybridize along their entire length. In particular, substantially complementary sequences may comprise a contiguous sequence of bases that do not hybridize to a target sequence, positioned 3' or 5' to a contiguous sequence of bases that hybridize under stringent hybridization conditions to a target sequence.

[0185] "Pharmaceutically acceptable" refers to a material that is not biologically or otherwise undesirable, i.e., the material may be incorporated into a pharmaceutical composition administered to a patient without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained. When the term "pharmaceutically acceptable" is used to refer to a pharmaceutical carrier or excipient, it is implied that the carrier or excipient has met the required standards of toxicological and manufacturing testing or that it is included on the Inactive Ingredient Guide prepared by the U.S. and Drug administration.

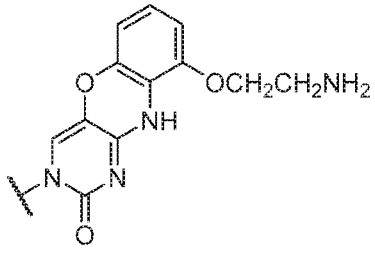
[0186] "Constructs" of the oligonucleotides can refer to an oligonucleotide of the present disclosure and, e.g., (1) a conjugated moiety, such as those described herein (such as targeting moieties) or (2) domains of modified/unmodified nucleotides, such as in some chimeric oligonucleotides.

[0187] "Chimeric oligonucleotide" refers to an oligonucleotide having more than one domain, for example, as exemplified by Formulae (VI) and (VII). The chimeric oligonucleotide may include additional components, e.g., a ligand-targeting group or a pharmacophore or additional nucleotides, linkers, etc.

[0188] "Modified nucleoside" refers to a nucleoside having, independently, a modified sugar moiety and/or modified nucleobase. It is understood that nucleosides can be linked through intersubunit linkages, such as phosphodiester intersubunit linkages, thiophosphate intersubunit linkages, phosphoramidate intersubunit linkages, and thiophosphoramidate intersubunit linkages "Modified nucleotides" may refer to a nucleoside and intersubunit linkage together.

[0189] "Unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). "Modified nucleobases" include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C≡C-CH_3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further modified nucleobases include tricyclic pyrimidines such as phenoxazine cytidine(1H-pyrimido[5,4-b][l,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido[5,4-b][l,4]benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (e.g. 9-(2-am-oelhoxy)-H-pyrimido[5,4-b][l,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5-b]indol-2-one), pyridoindole cytidine (H-pyrido[3,2 ,5]pyrrolo[2,3-d]pyrimidin-2-one). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine, and 2-pyridone.

[0190] In some embodiments, the modified nucleobase is selected from the group consisting of 5-methylcytosine, 2,6-diaminopurine, 5-methyluracil, and a g-clamp. In some embodiments, the g-clamp is



[0191] "Ligand targeting group" refers to a moiety that promotes delivery of the oligonucleotide to HBV infected hepatocytes through receptor binding. These groups include "receptor targeting ligands," such as GalNAc and Cholesterol, which target cell surface receptor ASGPR and LDL receptor on cell surfaces, respectively. Other receptor targeting ligands that target these receptors on cell surfaces are also within the scope of this term.

[0192] "Pharmacophore" refers to an oligonucleotide drug sequence that interacts HBV DNA or RNA molecules within HBV/HDV or HBV-infected cells and triggers antiviral responses.

[0193] "Conformationally restricted nucleoside" refers to nucleosides having a bridged or bicyclic sugar structure wherein the conformation of the nucleoside may be fixed in a particular configuration. For example, conformationally restricted nucleosides include those with fixed C_3'-endo sugar puckering. Exemplary embodiments include bridged nucleic acids (BNAs), e.g., 2', 4'-BNA nucleosides such as α-L-Methyleneoxy (4′-CH_2-O-2′) LNA, β-D-Methyleneoxy (4′-CH_2-O-2′) LNA, Ethyleneoxy (4′-$(CH_2)_2$-O-2′) ENA, 2',4'-BNA^{NC}[NH], 2',4'-BNA^{NC}[NMe], 2',4'-BNA^{NC}[NBn], aminooxy (4′-CH2—O—N(R)-2′) BNA, and oxyamino (4′-CH_2—N(R)—O-2′) BNA. Other exemplary BNA structures include but are not limited to, oligonucleotides having at least one bridge between the 4′ and the 2′ position of the sugar wherein each of the bridges independently comprises 1 or from 2 to 4 linked groups independently selected from —$[C(R_1)(R_2)]_n$—, —$C(R_1)$=$C(R_2)$—, —$C(R_1)$=N—, —C(=NR_1)—, —C(=O)—, —C(=S)—, —O—, —$Si(R_1)_2$—, —$S(=O)_x$— and —$N(R_1)$—; wherein: x is 0, 1, or 2; n is 1, 2, 3, or 4; each R_1 and R_2 is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12}alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, a heterocycle radical, a substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5-C_7 alicyclic radical, substituted C_5-C_7 alicyclic radical, halogen, OJ_1, NJ_1J_2, SJ_1, N_3, $COOJ_1$, acyl (C(=O)—H), substituted acyl, CN, sulfonyl ($S(=O)_2$-J_1), or sulfoxyl (S(=O)-J_1); and each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-

C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12}alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)—H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1-C_{12} aminoalkyl, substituted C_1-C_{12} aminoalkyl or a protecting group. Certain BNAs have been prepared and disclosed in the patent literature as well as in scientific literature (see for example: issued U.S. Pat. Nos. 7,053,207; 6,268,490; 6,770,748; 6,794,499; 7,034,133; 6,525,191; 7,696,345; 7,569,575; 7,314,923; 7,217,805; and 7,084,125, hereby incorporated by reference herein in their entirety. "Conformationally restricted nucleotide" refers to conformationally restricted nucleosides linked through an intersubunit linkage.

[0194] In some embodiments, the conformationally restricted nucleoside is selected from optionally substituted LNA or optionally substituted ENA. The optionally substituted LNA or ENA may be substituted by an alkyl moiety, for example a methyl or ethyl on one of the $-CH_2-$ moieties.

[0195] "Inhibiting expression" refers to a reduction or blockade of the expression or activity and does not necessarily indicate a total elimination of expression or activity.

[0196] "Inhibiting replication of a virus" refers to reduction or blockade of the replication of a virus and does not necessarily indicate a total elimination of replication of the virus.

[0197] "Subject" refers to mammals and includes humans and non-human mammals. In some embodiments, the subject is a human, such as an adult human.

[0198] "Treating" or "treatment" of a disease in a subject refers to (1) preventing the disease from occurring in a subject that is predisposed or does not yet display symptoms of the disease; (2) inhibiting the disease or arresting its development; or (3) ameliorating or causing regression of the disease.

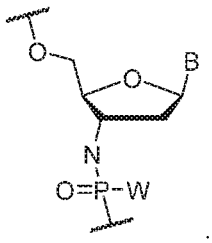
[0199] "Therapeutically effective amount" means an amount of a pharmaceutical agent that provides a therapeutic benefit to a subject.

[0200] "Pharmaceutically acceptable salt" means physiologically and pharmaceutically acceptable salts of the compounds of the present disclosure, i.e., salts that retain the desired biological activity of the parent oligonucleotide/compound and do not impart undesired toxicological effects thereto.

[0201] The following abbreviations are used in this disclosure. 2'-H (deoxyribose) nucleosides are referred to by an uppercase letter corresponding to the nucleobase, e.g., A, C, G, and T. 2'-OH (ribose) nucleosides are referred to by a lowercase r and an uppercase letter corresponding to the nucleobase, e.g., rA, rC, rG, and rU. 2'-O-Me nucleosides are referred to by a lowercase m and an uppercase letter corresponding to the nucleobase, e.g., mA, mC, mG and mU. 2'-MOE nucleosides are referred to by a lowercase "moe" and an uppercase letter corresponding to the nucleobase, e.g., moeA, moeC, moeG and moeU. 2'-ribo-F nucleosides are referred to by a lowercase "f" and an uppercase letter corresponding to the nucleobase, e.g., fA, fC, fG and fU. 2'-arabino-F nucleosides are referred to by a lowercase "af" and an uppercase letter corresponding to the nucleobase, e.g., afA, afC, afG and afU. mA* is 3'-amino-2'-OMe-2,6-Diaminopurine. A* is 3'-amino-2'-deoxy-2,6-Diaminopurine. fA* is 3'-amino-2'-F-2,6-Diaminopurine. LNA nucleosides are referred to by an "L" and an uppercase letter corresponding to the nucleobase, e.g., LA, LC, LG, LT.

[0202] For the backbone or intersubunit linkages of the nucleotides, phosphodiester intersubunit linkages are referred to as "PO" or are generally not included in sequence details; thiophosphate intersubunit linkages are abbreviated as lowercase "ps"; phosphoramidate intersubunit linkages are abbreviated as lowercase "np"; and thiophosphoramidate intersubunit linkages are abbreviated as lowercase "nps."

[0203] N3'→P5' refers to modified nucleotides having intersubunit linkages where the 3' moiety contains N (e.g., NH) and is linked through a P. For example, the following structure has a N3'→P5' linkage:



[0204] It is noted that, as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is

intended to serve as antecedent basis for use of such exclusive terminology as "solely", "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0205] The term "about" will be understood by persons of ordinary skill in the art and will vary to some extent depending upon the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, "about" will mean up to plus or minus 10% of the particular term. Certain ranges are presented herein with numerical values being preceded by the term "about". The term "about" is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number, which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number.

[0206] It is also to be appreciated that the various modes of treatment or prevention of the diseases or conditions described herein are intended to mean "substantial," which includes total but also less than total treatment or prevention, and wherein some biologically or medically relevant result is achieved. The treatment may be a continuous prolonged treatment for a chronic disease or a single, or few time administrations for the treatment of an acute condition.

[0207] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0208] This disclosure is not limited to particular embodiments described, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0209] As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present invention. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

[0210] All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates that may need to be independently confirmed.

5. Examples

[0211] The following examples illustrate certain embodiments of the present disclosure to aid the skilled person in practicing the disclosure. Accordingly, the examples are in no way considered to limit the scope of the disclosure.

Methods of making

[0212] All the monomers were dried in vacuum desiccator with desiccants (KOH and P_2O_5, RT 24h). Synthesis solid supports (CPG) attached to the first 5′ residue were obtained from commercially available sources. All other synthesis reagents and solvents were obtained from commercially available sources and used as such. The chemicals and solvents for post synthesis workflow were purchased from commercially available sources and used without any purification or treatment. Solvent (Acetonitrile) and solutions (amidite and activator) were stored over molecular sieves during synthesis.

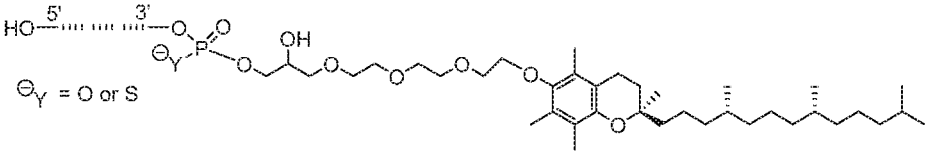
[0213] The control, nuclease stabilized, 3'-cholesterol, 3'-Tocopherol and 3'-GalNAc conjugated antisense oligonucleotides used in this study are shown, e.g., in Tables 10-13. The antisense oligonucleotides were synthesized on an ABI-394 synthesizer using the standard 93-

step cycle written by the manufacturer. The solid support was controlled pore glass and the monomers contained standard protecting groups. Each oligonucleotide was individually synthesized using commercially available 5'-*O*-(4,4'-dimethoxytrityl)-3'-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl) DNA and or 2'-O-Me phosphoramidite monomers of 6-*N*-benzoyladenosine (A^{Bz}), 4-*N*-acetylcytidine (C^{Ac}), 2-*N*-isobutyrylguanosine (G^{iBu}), and Thymidine (T), according to standard solid phase oligonucleotide synthesis protocols. The phosphoramidites were purchased from commercially available sources. The 2'-O-Me-2,6,diaminopurine phosphoramidite was purchased from commercially available sources. The DDTT ((dimethylamino-methylidene) amino)-3H-1,2,4-dithiazaoline-3-thione was used as the sulfur-transfer agent for the synthesis of oligoribonucleotide phosphorothioates. Modified oligonucleotides were obtained using an extended coupling of 0.1M solution of phosphoramidite in CH_3CN in the presence of 5-(ethylthio)-1*H*-tetrazole activator to a solid bound oligonucleotide followed by standard capping, oxidation and deprotection. The stepwise coupling efficiency of all modified phosphoramidites was more than 98%. Oligonucleotide-bearing solid supports were heated with aqueous ammonia/ethanol (3:1) solution at 55 °C for 8 h to deprotect the base labile protecting groups.

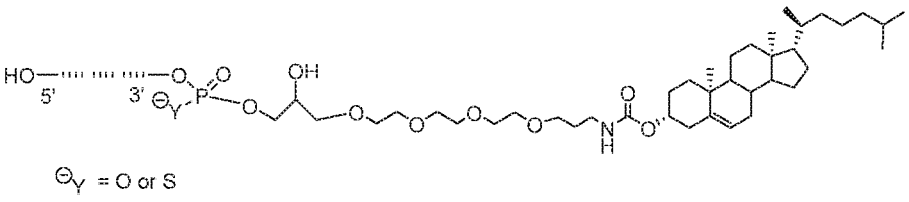
[0214] The cholesterol and tocopherol conjugated oligonucleotides were obtained by starting solid phase synthesis on cholesterol and Tocopherol support attach on TEG linker and final coupling of the phosphoramidite to the support-bound oligonucleotide. The GalNAc conjugated ASOs were synthesized from a hydroxyprolinol-GalNAc solid support. GalNAc was tethered to *trans*-4-hydroxyprolinol via a 6-aminohexanoate linkage to obtain a hydroxyprolinol-GalNAc moiety that was subsequently attached to a functionalized control pore glass (CPG) to obtain the solid support.

[0215] The unconjugated and GalNAc modified oligonucleotides were purified by anion-exchange HPLC. The buffers were 20 mM sodium phosphate in 10 % CH_3CN, pH 8.5 (buffer A) and 20 mM sodium phosphate in 10% CH_3CN, 1.8 M NaBr, pH 8.5 (buffer B). Fractions containing full-length oligonucleotides were pooled, desalted and lyophilized.

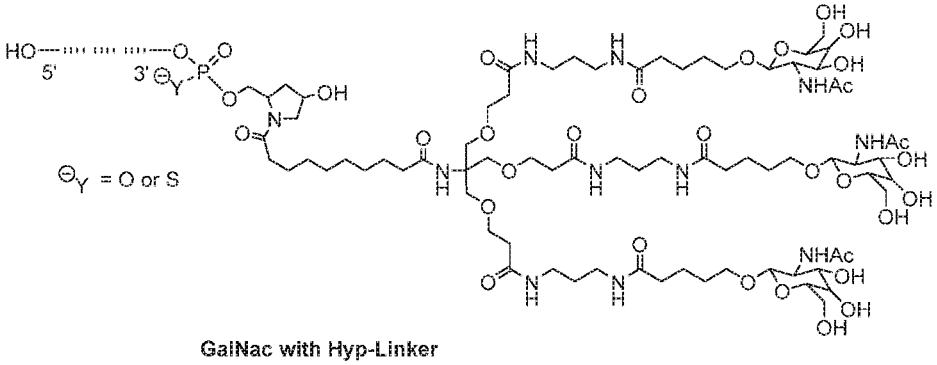
[0216] The cholesterol and tocopherol conjugated sequences were purified by high-performance liquid chromatography (HPLC) on an in-house packed RPC-Source15 reverse-phase column. The buffers were 20 mM NaOAc in 10 % CH_3CN (buffer A) and 20 mM NaOAc in 70% CH_3CN (buffer B). Analytical HPLC and ES LC-MS established the integrity of the oligonucleotides.



Vit E TEG linker



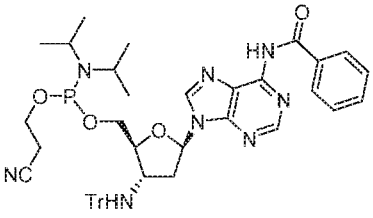
Cholesterol with TEG Linker



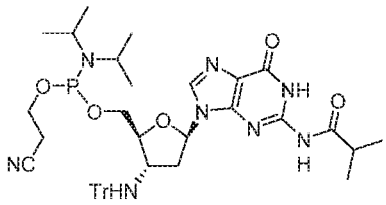
GalNac with Hyp-Linker

Synthesis of Phosphoramidate (NP) and Thiophosphoramidate (NPS) Modified Oligonucleotides

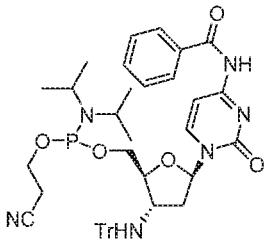
[0217] The NP and NPS modified oligonucleotides were synthesized on an ABI-394 synthesizer using the 93-step cycle written with modifications to deblock, coupling and wait steps. The solid support was 3'-NHTr-5'-LCAA-CPG. Each oligonucleotide was individually synthesized using 3'-NH-Tr-5'-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl) DNA phosphoramidite monomers of 6-*N*-benzoyladenosine (A^{Bz}), 4-*N*-Benzylcytidine (C^{Bz}), 2-*N*-isobutyrylguanosine (G^{iBu}), and Thymidine (T), according to standard solid phase phosphoramidite chemistry protocols by using the procedure described in *Nucleic Acids Research, 1995, Vol. 23, No. 14 2661-2668.*



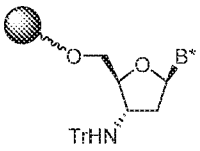
3'-NHTr-dA (Bz)



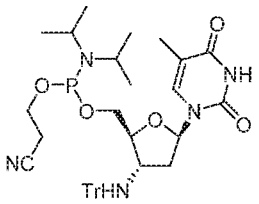
3'-NHTr-dG(iBu)



3'-NHTr-dC(Bz)



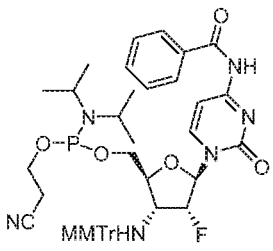
B* = A, C, G or T



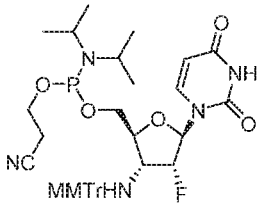
3'-NHTr-T:

3'-NHTr-DNA building blocks for oligomer synthesis

[0218] The 2'-F 3'-NH-MMTr-5'-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl) Uridine (U) and 4-*N*-benzoylcytidine (C^{Bz}) phosphoramidite monomers) were synthesized by using the procedure described in *Nucleic Acids Research, 1996, Vol. 24, No. 15, **2966–2973***

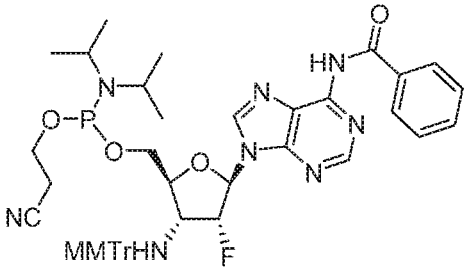


3'-NHMMTr-2'-F-C(Bz)

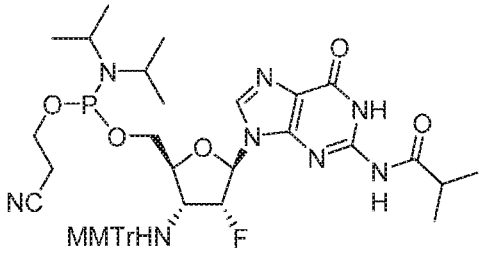


3'-NHMMTr-2'-F U:

2'-F 3'-NH-MMTr-5'-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl) 6-*N*-benzoyladenosine (A^{Bz}), 2-*N*-isobutyrylguanosine (G^{iBu}), were synthesized as the procedure described below

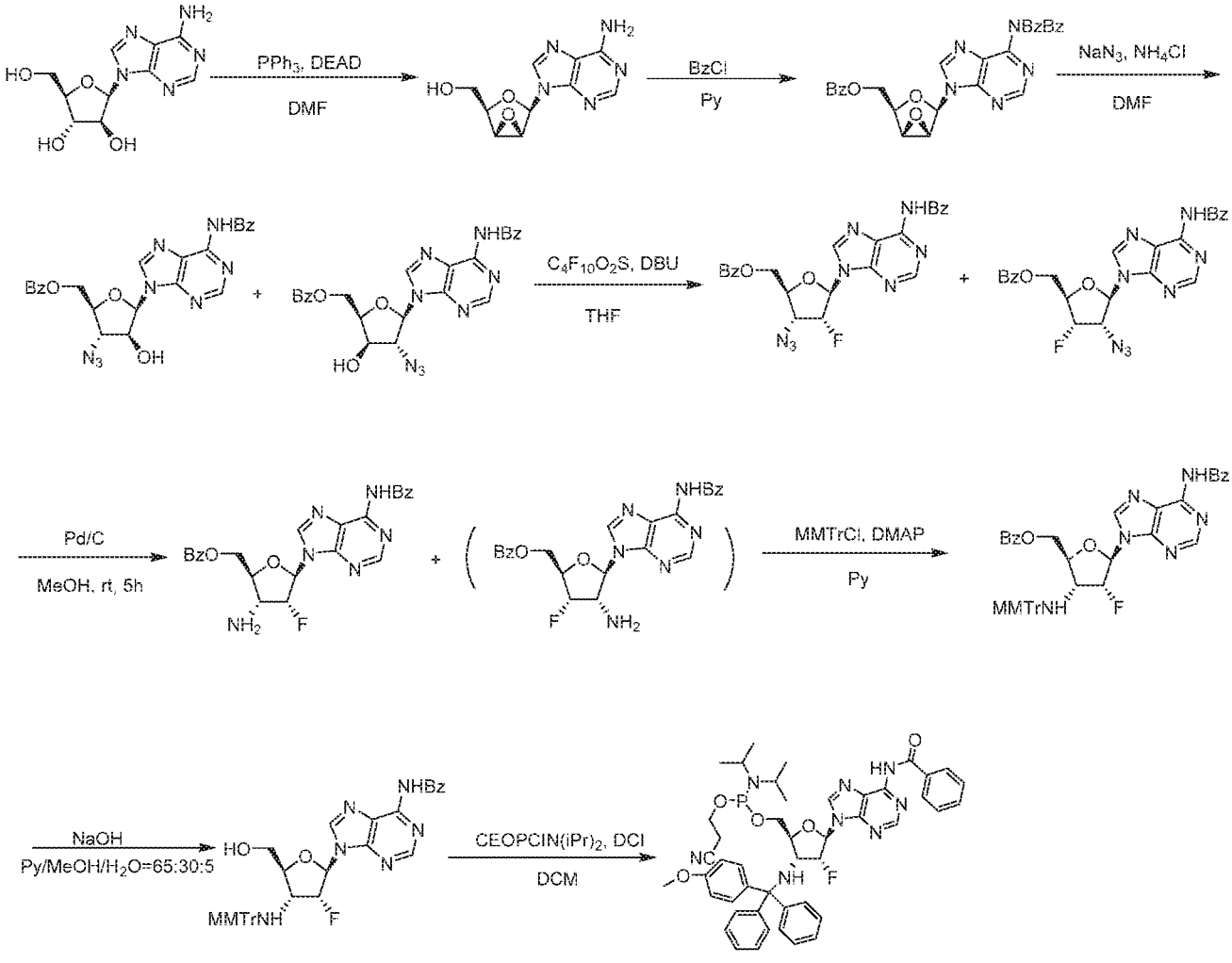


3'-NHMMTr-2'-F A (Bz)

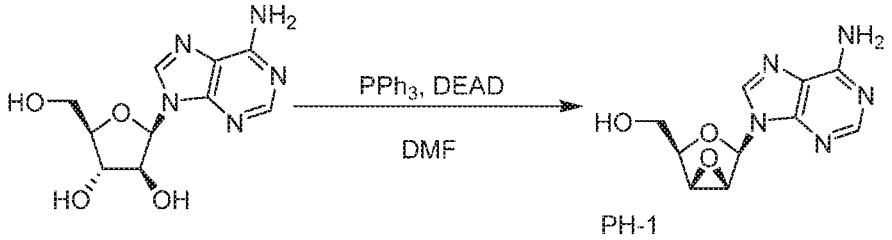


3'-NHMMTr-2'-F G(iBu)

**



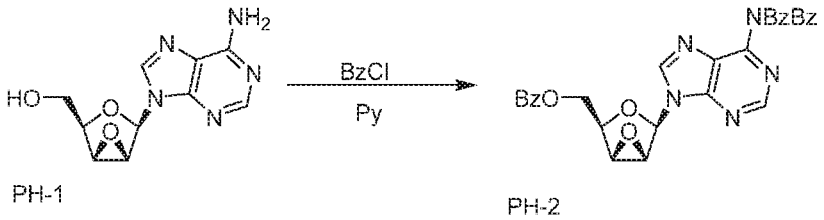
Preparation of PH-1



PH-1

[0219] To a solution of (2R,3S,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol (300 g, 1.123 mol, 1.00equiv) in N,N-dimethylformamide (7500mL) with an inert atmosphere of nitrogen, was added triphenylphosphine (735 g, 2.802 mol, 2.50equiv).The resulting solution was stirred for 15 min at 0°C. This was followed by the addition of a solution of diethyl azodicarboxylate (449.4 g, 2.581 mol, 2.54 equiv.) in N, N-dimethylformamide (7500 mL) dropwise with stirring at 0°C in 60 min. The resulting solution was stirring, for 2 h at 25°C. The resulting mixture was concentrated under reduced pressure. The product was precipitated by the addition of ether. The solids were collected by filtration. The crude product was purified by re-crystallization from methanol. The solid was dried in an oven under reduced pressure. This resulted in 186 g (66%) of **PH-1** as a white solid. 1H-NMR (DMSO-d_6, 400MHz): 8.34 – 8.07 (m, 2H), 7.44 – 7.26 (m, 2H), 6.30 – 6.21 (m, 1H), 5.07 – 4.95 (m, 1H), 4.33 – 4.20 (m, 1H), 4.15 – 4.03 (m, 2H), 3.71 – 3.50 (m, 2H).

Preparation of PH-2

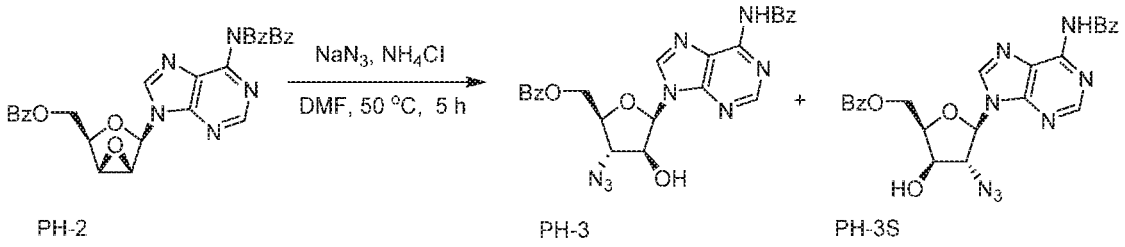


PH-1 PH-2

[0220] To a solution of **PH-1** (100g, 401.2 mmol, 1.00 equiv.) in pyridine (1000 mL) with an inert atmosphere of nitrogen, was added benzoyl chloride (175 g, 1.245 mol, 3.10 equiv.) dropwise with stirring at 0°C in 30 min. The resulting solution was stirred for 3 h at 25°C. The resulting solution was diluted with 400 mL of ethyl acetate. The resulting mixture was washed with 3x300 mL of water and 2x300 mL of saturated sodium bicarbonate solution respectively. The resulting mixture was washed with 1x300 mL of saturated sodium chloride solution. The mixture was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced

pressure. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (2/1). This resulted in 157 g (70%) of **PH- 2** as a white solid.

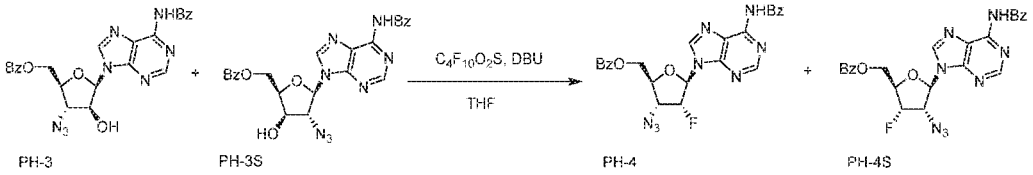
Preparation of PH-3



PH-2 PH-3 PH-3S

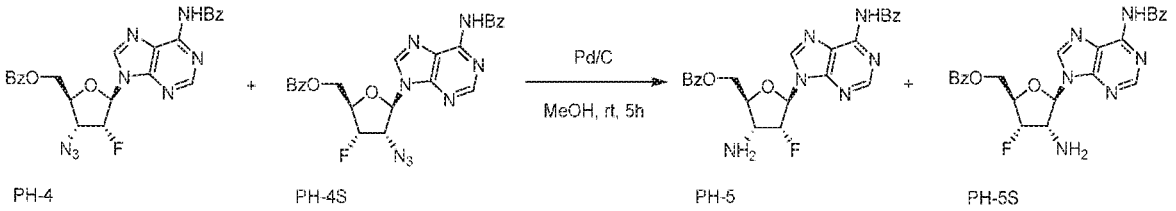
[0221] To a solution of **PH-2** (30 g, 53.42mmol, 1.00equiv) in N,N-dimethylformamide (300 mL) with an inert atmosphere of nitrogen, was added ammonium chloride (5.7 g, 106.56mmol, 2.00equiv) and sodium azide (34.8 g, 535.30mmol, 10.00equiv) in order. The resulting solution was stirred for 5 h at 50°C. The resulting solution was diluted with 2000 mL of dichloromethane. The resulting mixture was washed with 3x2000 mL of water, 1x2000 mL of saturated sodium bicarbonate solution and 1x2000 mL of saturated sodium chloride solution respectively. The mixture was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. This resulted in 24 g (90%) of **PH-3** and **PH-3S** (5:1) as a white solid.

Preparation of PH-4



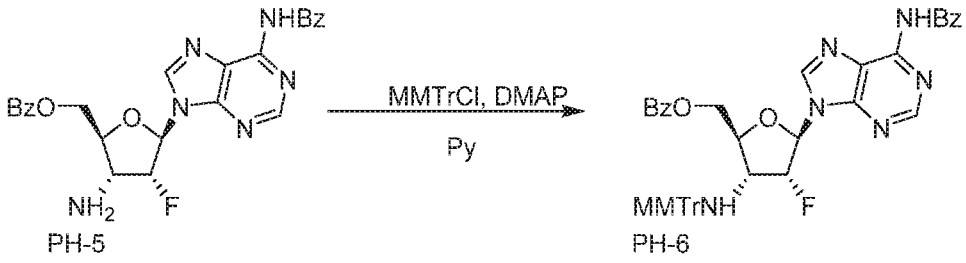
PH-3 PH-3S PH-4 PH-4S

[0222] To a solution of **PH- 3** and **PH-3S** (5:1) (10 g, 19.98mmol, 1.00equiv) in tetrahydrofuran (100mL) with an inert atmosphere of nitrogen, was added 1, 8-Diazabicyclo [5.4.0] undec-7-ene (10.69 g, 70.22mmol, 3.50equiv). This was followed by the addition of perfluorobutylsulfonyl fluoride (12.69 g, 2.10equiv) dropwise with stirring at 0°C in 10 min. The resulting solution was stirred for 1.5 h at 0°C. The resulting solution was diluted with 200 mL of dichloromethane. The resulting mixture was washed with 3x200 mL of water, 1x200 mL of saturated sodium bicarbonate solution and 1x200 mL of saturated sodium chloride solution respectively. The mixture was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was re-crystallized from ethyl acetate/petroleum ether in the ratio of 1:1. This resulted in 6 g (60%) of **PH-4** and **PH-4S** (5:1) as a white solid. MS m/z [M+H]+ (ESI): 503.



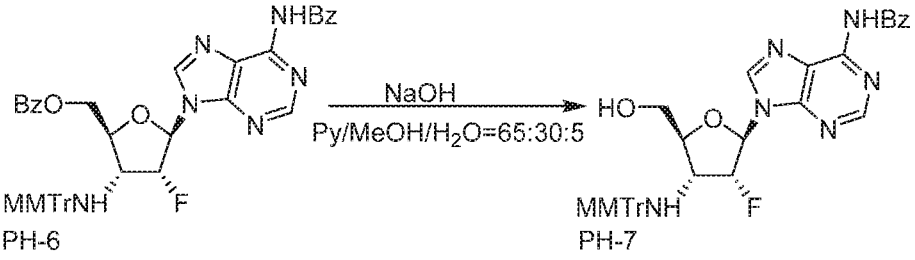
[0223] To a solution of **PH-4** and **PH-4S** (5:1) (10 g, 19.90mmol, 1.00equiv) in tetrahydrofuran (150 mL), was added 10 % palladium carbon (3.0 g). The flask was evacuated and flushed three times with nitrogen, followed by flushing with hydrogen. The resulting solution was stirred for 1 h at room temperature. The solids were filtered out. The resulting mixture was concentrated under reduced pressure. The crude product (10 g) was purified by Flash-Prep-HPLC with the following conditions (IntelFlash-1): Column, C18; mobile phase, waters and acetonitrile (30% acetonitrile up to 50% in 30 min); Detector, UV 254 nm. This resulted in 7 g (74%) of **PH-5** as a white solid and 1.0g of **PH-5S** as a white solid. MS m/z [M+H]+ (ESI): 477.

Preparation of PH-6



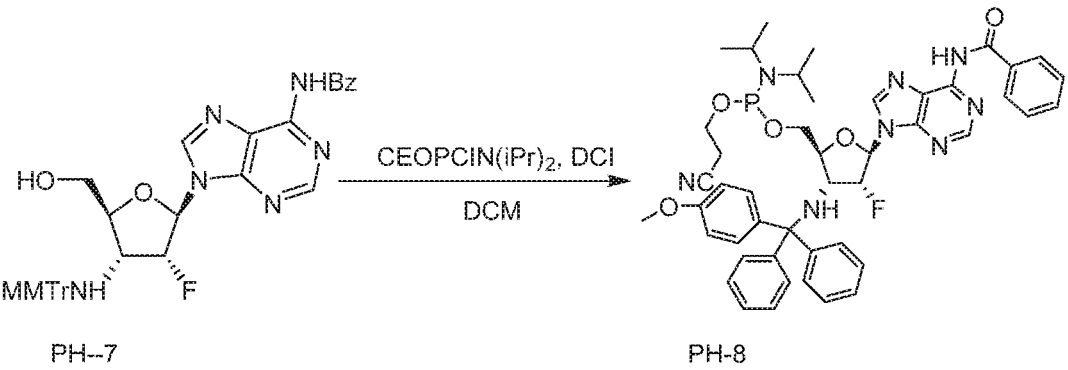
[0224] To a solution of **PH-5** (4 g, 8.40mmol, 1.00equiv) in pyridine (40 mL) with an inert atmosphere of nitrogen, was added 4-dimethylaminopyridine (1.5 g, 12.28mmol, 1.50equiv) and 4-methoxytriphenylmethyl chloride (10.3 g, 4.00equiv) in order. The resulting solution was stirred for 16 h at 25°C. The resulting solution was diluted with 300 mL of dichloromethane. The resulting mixture was washed with 1x300 mL of water and 3x300 mL of saturated sodium bicarbonate solution. The resulting mixture was washed with 1x300 mL of saturated sodium chloride solution respectively. The mixture was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was applied onto a silica gel column with dichloromethane /methanol (100/1). This resulted in 5.7 g (91%) of **PH-6** as a white solid.

Preparation of PH-7



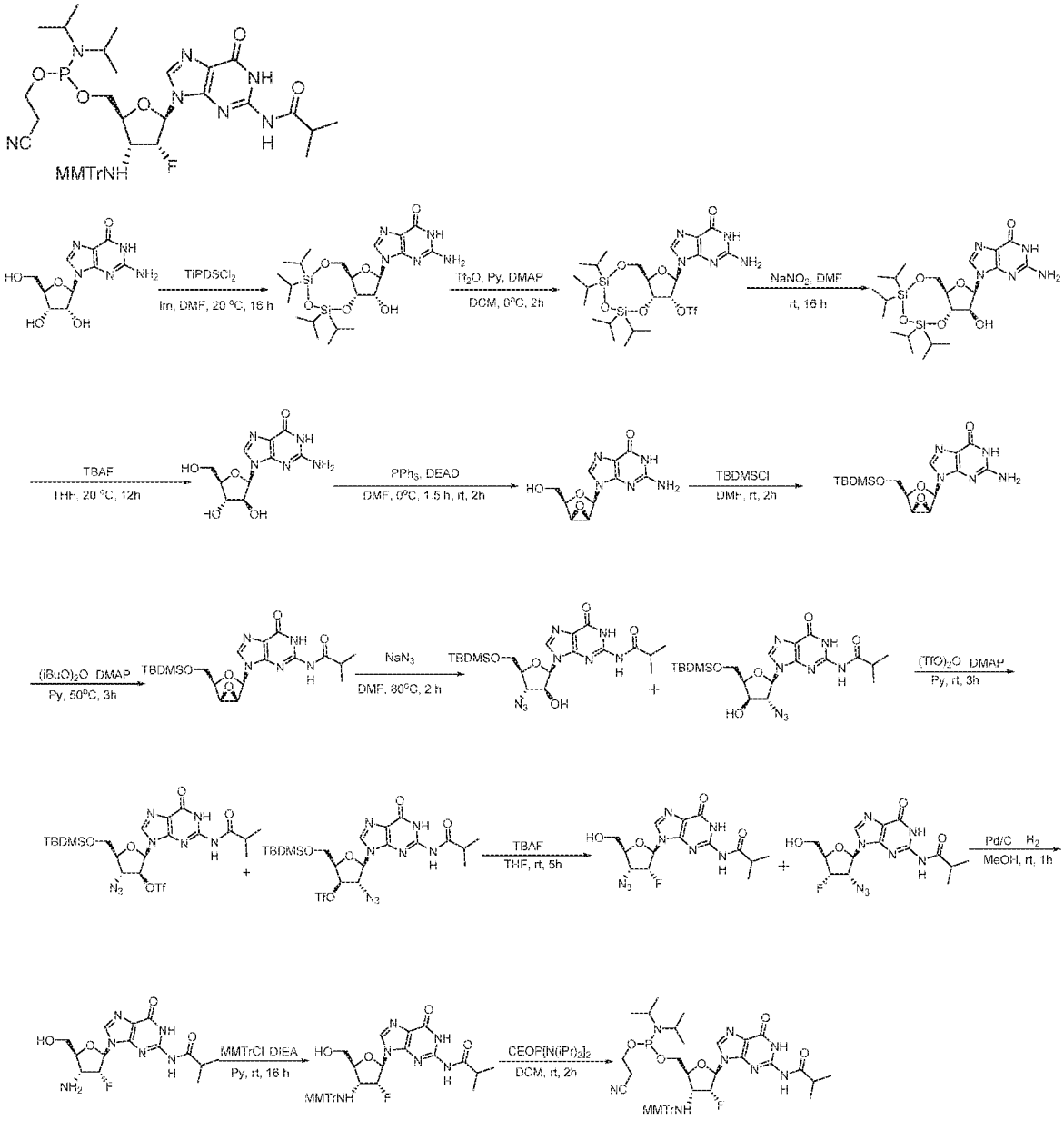
[0225] To a solution of **PH-6** (5g, 6.68mmol, 1.00equiv) in pyridine/methanol/water (32.2/14.7/2.4 mL), was added sodium hydroxide (2 mol/L) (7.2 mL, 1.10equiv) dropwise with stirring at 0°C in 5min. The resulting solution was stirred for 20 min at 0°C. The reaction was then quenched by the addition of 200 mL of ice water. The resulting solution was extracted with 400 mL of dichloromethane and the organic layers combined. The resulting mixture was washed with 1x300 mL of water and 1x300 mL of saturated sodium chloride solution. The mixture was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was applied onto a silica gel column with methanol/ dichloromethane (1:100). This resulted in 4.3 g (100%) of **PH-** 7 as a white solid. MS m/z [M+H]+ (ESI): 645.

Preparation of PH-8

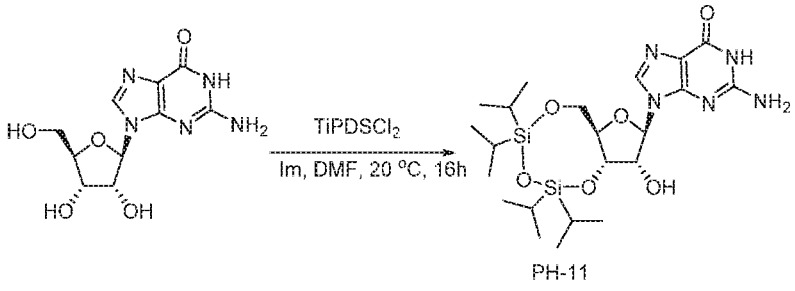


[0226] To a solution of **PH-** 7 (19.4g, 35.89mmol, 1.00equiv) in dichloromethane (200 mL) with an inert atmosphere of nitrogen, was added3-([bis [bis (propan-2-yl) amino] phosphanyl] oxy) propanenitrile (11.79g, 39.12mmol, 1.30equiv). This was followed by the addition of 4, 5-Dicyanoimidazole (4.26 g, 1.20equiv) at 0°C. The resulting solution was stirred for 30 min at room temperature. The resulting solution was diluted with 1000 mL of dichloromethane. The resulting mixture was washed with 3x800 mL of saturated sodium bicarbonate solution and 1x800 mL of sodium chloride solution respectively. The mixture was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was

purified by Flash-Prep-HPLC with the following conditions: Column, C18; mobile phase, waters and acetonitrile (40% acetonitrile up to 80% in 6 min); Detector, UV 254 nm. This resulted in 15.2 g (50%) of **PH-8** as a white solid. MS m/z [M+H] + (ESI): 845.

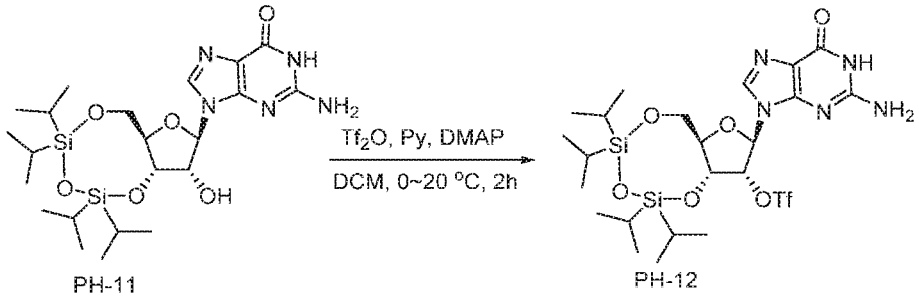


Preparation of PH-11



[0227] To a solution of 2-amino-9-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6,9-dihydro-1H-purin-6-one (700 g, 2.47mol, 1.00equiv) in N,N-dimethylformamide (7 L) with an inert atmosphere of nitrogen, was added imidazole (504 g, 7.41mol, 3.00equiv).This was followed by the addition of 1, 3-Dichloro-1, 1, 3, 3-tetraisopropyldisiloxane (770 g, 2.44 mol, 1.00equiv) dropwise with stirring at 20°C. The resulting solution was stirred for 16 h at 20°C. The reaction solution was then poured into 70L of water/ice. The solids were collected by filtration. This resulted in 1200 g (92%) of **PH-11** as a white solid. MS m/z [M+H] + (ESI): 526.

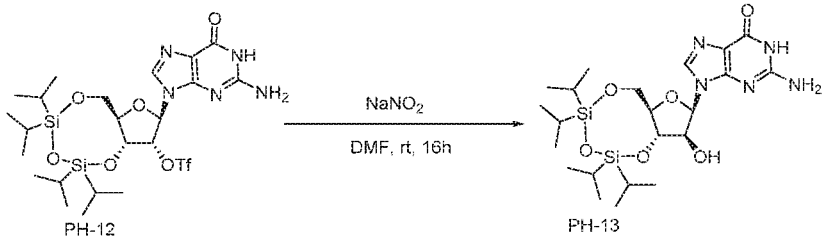
Preparation of PH-12



[0228] To a solution of **PH-11** (530 g, 1.01mol, 1.00equiv) in dichloromethane (5000 mL) with an inert atmosphere of nitrogen, was added pyridine (725 g, 9.17mol, 9.00equiv) and 4-dimethylaminopyridine (147 g, 1.20mol, 1.20equiv) in order. This was followed by the addition of trifluoromethanesulfonic anhydride (426 g, 1.51mol, 1.20equiv) dropwise with stirring at 0°C. The resulting solution was stirred for 15 min at 0°C. Then the resulting solution was allowed to react with stirring, for an additional 2 h at 20°C. The resulting solution was diluted with 5000 mL of dichloromethane. The resulting solution was washed with 2x3000 mL of saturated sodium bicarbonate and 1x3000 mL of saturated sodium chloride respectively. The solution was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. This resulted in 600 g (90%) of **PH- 12** as a brown solid.

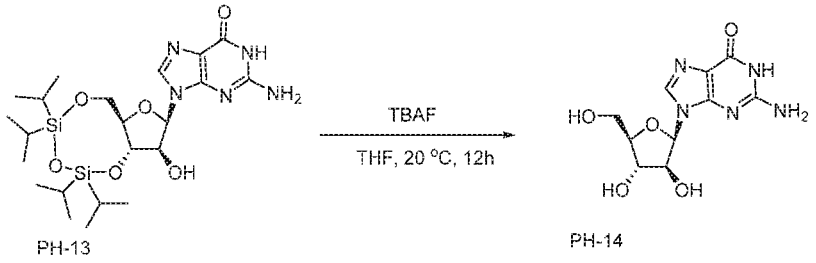
The product was used in the next step directly without further purification.

Preparation of PH-13



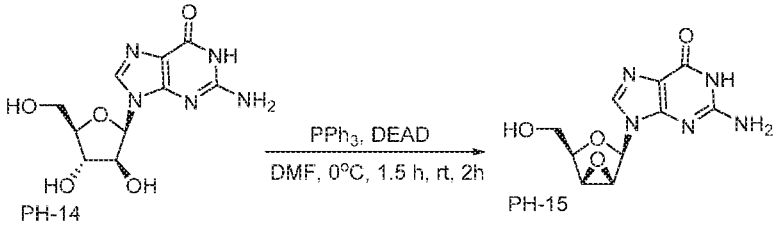
[0229] To a solution of **PH-12** (200 g, 304.04mmol, 1.00equiv) in N,N-dimethylformamide (1000 mL) with an inert atmosphere of argon, was added sodium nitrite (115 g, 1.67mol, 5.00equiv). The resulting mixture was stirred for 16 h at 25°C. The resulting solution was poured into 5000 ml water/ice. The solids were collected by filtration. The crude product was re-crystallized from dichloromethane/acetonitrile in the ratio of 1/4 (50 ml/g). This resulted in 78 g (49% over last two steps) of **PH-13** as a solid. MS m/z [M+H] + (ESI): 526.

Preparation of PH-14



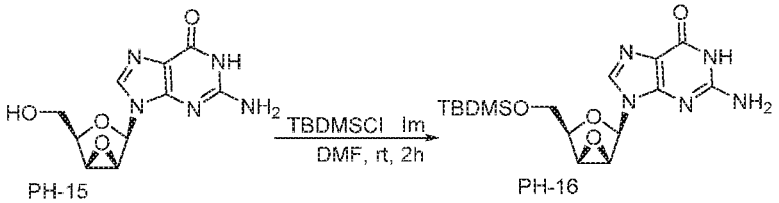
[0230] To a solution of **PH- 13** (50 g, 95.10mmol, 1.00equiv) in tetrahydrofuran (500 mL) with an inert atmosphere of nitrogen, was added tetrabutylammonium fluoride (95 mL, 1.00equiv, 1N in tetrahydrofuran). The resulting mixture was stirred for 12 h at 20°C. The resulting mixture was concentrated under reduced pressure. The crude was re-crystallized from methanol/ethyl acetate in the ratio of 1/5 (20 ml/g) three times. The solids were collected by filtration, and then purified by Flash with the following conditions: Column, C18 silica gel; mobile phase, waters and acetonitrile (2% acetonitrile up to 10% in 10 min); Detector, UV 254 nm. This resulted in 16 g (59%) of **PH- 14** as a brown solid. 1H-NMR (DMSO-d_6, 400MHz): 10.44(s, 1H), 6.49(s, 2H), 6.02(s, 1H), 5.55-5.65(m, 2H), 5.10(s, 1H), 4.08(m, 2H), 3.76(m, 1H), 3.64(m, 1H).

Preparation of PH-15



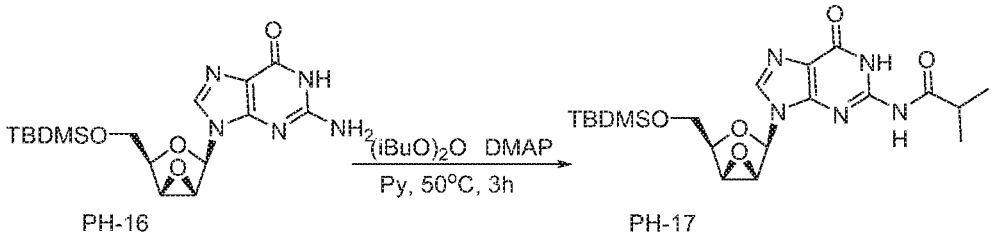
[0231] To solution of **PH-14** (220 g, 776.72mmol, 1.00equiv) in N,N-dimethylformamide (2000 mL) with an inert atmosphere of argon, was added triphenylphosphine (509 g, 1.94mol, 2.50equiv). The resulting solution was stirred for 1.5 h at 0°C. To this was added diethyl azodicarboxylate (338 g, 1.94mol, 2.50equiv) dropwise with stirring at 0°C. The resulting solution was stirred for 2 h at room temperature. The resulting mixture was poured into 20 L cold ethyl ether. The solids were collected by filtration, then re-crystallized from methanol/ ethyl acetate in the ratio of 1/10 (10 ml/g). This resulted in 100 g (49%) of **PH-15** as a brown solid. MS m/z [M+H]+ (ESI): 266.

Preparation of PH-16



[0232] To a solution of **PH-15** (100 g, 377.0 mmol, 1.00equiv) in N,N-dimethylformamide (1000 mL) with an inert atmosphere of nitrogen, was added imidazole (77 g, 1.131 mol, 3.00equiv). This was followed by the addition of tert-butyldimethylsilyl chloride (142 g, 942 mmol, 1.50 equiv.) dropwise with stirring at 0°C. The resulting solution was stirred for 2 h at room temperature. The reaction was then quenched by the addition of methanol. The resulting mixture was concentrated under reduced pressure. The residue was applied onto a silica gel column with dichloromethane/methanol (100:1~15:1). This resulted in 80 g (85%) of **PH-16** as a solid. MS m/z [M+H]+ (ESI): 380.

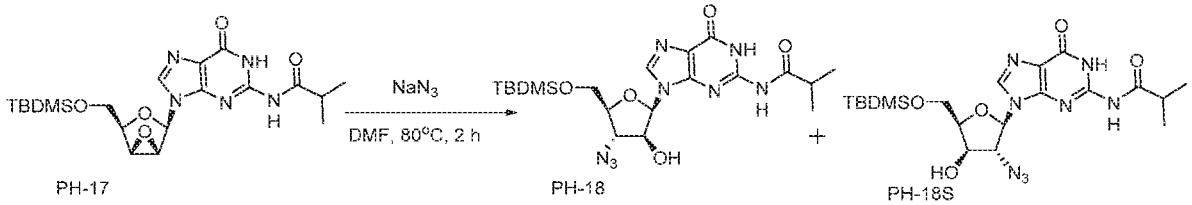
Preparation of PH-17



PH-16 PH-17

[0233] To a solution of **PH-16** (73 g, 192.37mmol, 1.00equiv) in pyridine (730 mL) with an inert atmosphere of nitrogen, was added 4-dimethylaminopyridine (23.5 g, 192.35mmol, 0.50equiv). This was followed by the addition of isobutyric anhydride (213 g, 1.35mol, 5.00equiv) dropwise with stirring. The resulting solution was stirred for 3 h at 50°C. The reaction was then quenched by the addition of ice water. The resulting solution was extracted with 3x2000 mL of dichloromethane and the organic layers combined. The resulting mixture was washed with 3x2000 mL of saturated sodium bicarbonate, 3x2000 mL of water and 3x2000 mL of saturated sodium chloride respectively. The organic layers was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was applied onto a silica gel column with dichloromethane/methanol (100:1~20:1). This resulted in 52 g (60%) of **PH-17** as a yellow solid. MS m/z [M+H]+ (ESI): 450.

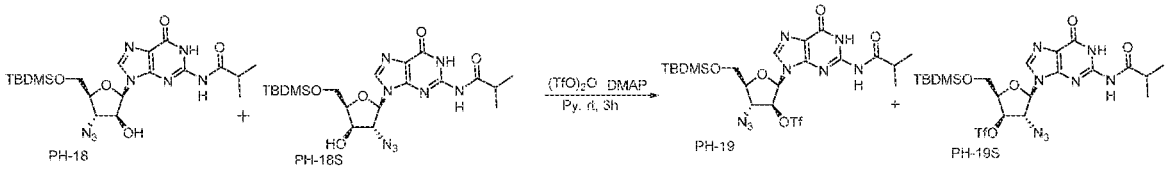
Preparation of PH-18



PH-17 PH-18 PH-18S

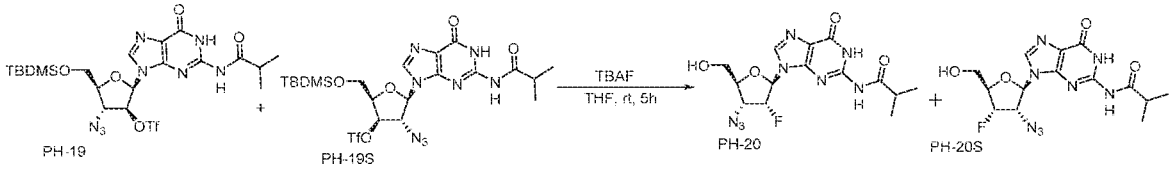
[0234] To a solution of **PH-17** (20 g, 44.4mmol, 1.00equiv) in N, N-dimethylformamide (100 mL) with an inert atmosphere of nitrogen was added sodium azide (18 g, 267mmol, 6.00equiv). The resulting solution was stirred for 2 h at 80°C. The resulting mixture was diluted with 1000 mL of dichloromethane. The resulting solution was washed with 3x1000 mL of saturated sodium bicarbonate, 3x1000 mL of water and 3x1000 mL of saturated sodium chloride respectively. The solution was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was applied onto a silica gel column with dichloromethane/methanol (100/1~40/1).This resulted in 11 g (50%) of **PH-18/PH-18S** (5.2:1) as a yellow solid. MS m/z [M+H]+ (ESI): 493

Preparation of PH-19



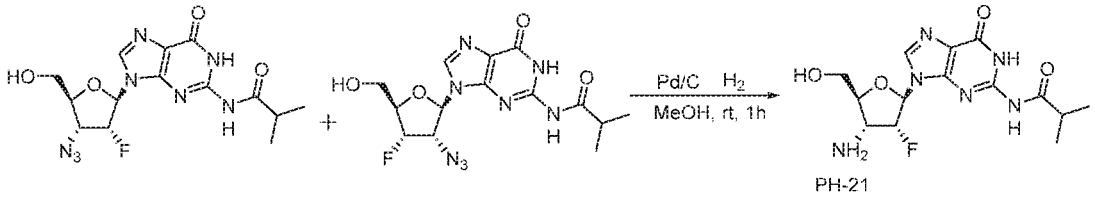
[0235] To a solution of **PH-18/PH-18S** (5.2:1) (16 g, 37.87mmol, 1.00equiv) in dichloromethane (160 mL), was added pyridine (23 g, 290.77mmol, 9.00equiv) and dimethylaminopyridine (4.35 g, 35.66mmol, 1.20equiv). This was followed by the addition of 1, 3-bis (trifluoromethylsulfonyl)trioxidane (11.9 g, 37.88mmol, 1.20equiv) dropwise with stirring at 0°C. The resulting solution was stirred for 2 h at 20°C. The reaction was quenched by the addition of water/ice. The resulting mixture was extracted with 2x1000 mL of dichloromethane and the organic layers combined. The resulting solution was washed with 1x1000 mL of saturated sodium chloride. The resulting solution was concentrated under reduced pressure. This resulted in 16 g (68%) of **PH-19/PH-19S** as a brown solid. The product was used in the next step directly without further purification.

Preparation of PH-20



[0236] To a solution of **PH-19/PH-19S** (16 g, 25.61mmol, 1.00equiv) in tetrahydrofuran (160 mL) with an inert atmosphere of argon, was added tetrabutylammonium fluoride (100 mL, 5.00equiv) dropwise with stirring at 0°C. The resulting solution was stirred for 5 h at room temperature. The resulting solution was diluted with 1000 mL of dichloromethane. The resulting solution was washed with 1x500 mL of water and 1x500 mL of saturated sodium chloride respectively. The resulting solution was concentrated under reduced pressure. The residue was applied onto a silica gel column with dichloromethane/methanol (100/1~20/1). This resulted in 8 g (85%) of **PH-20/PH-20S** (7:1) a yellow solid. MS m/z [M+H]+ (ESI): 381.

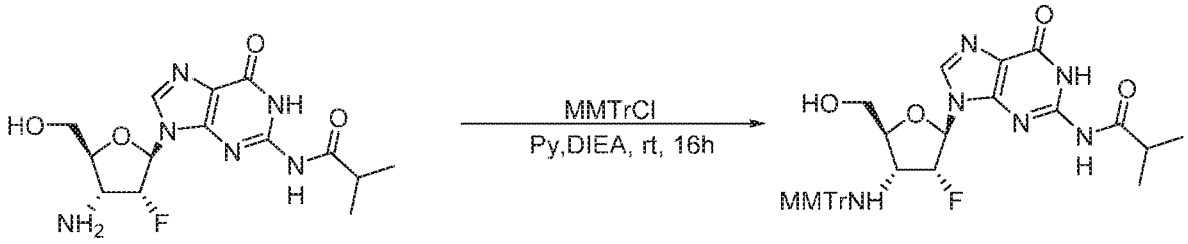
Preparation of PH-21



PH-21

[0237] To a solution of **PH-20/PH-20S** (3.4 g, 8.94mmol, 1.00equiv) in methanol (50 mL) was added 10 % palladium carbon (1.7 g). The flask was evacuated and flushed three times with nitrogen, followed by flushing with hydrogen. The resulting solution was stirred for 1 h at room temperature. The resulting solution was diluted with 100 mL of methanol. The solids were filtered out. The resulting solution was concentrated under reduced pressure. The crude product was purified by Flash-Prep-HPLC with the following conditions: Column, C18 silica gel; mobile phase, waters and acetonitrile (5% acetonitrile up to 50% in 35 min); Detector, UV 254 nm. This resulted in 1.7 g (54%) of **PH-21** as a white solid. 1H-NMR (DMSO-d_6, 400MHz): 12.13 (s, 1H), 11.91 (s, 1H), 8.91 (s, 2H), 8.23 (s, 2H), 7.25 (m, 1H), 5.78 (m, 1H), 4.62-3.72 (m, 4H), 2.92 (m, 1H), 1.13 (s, 6H).

Preparation of PH-22

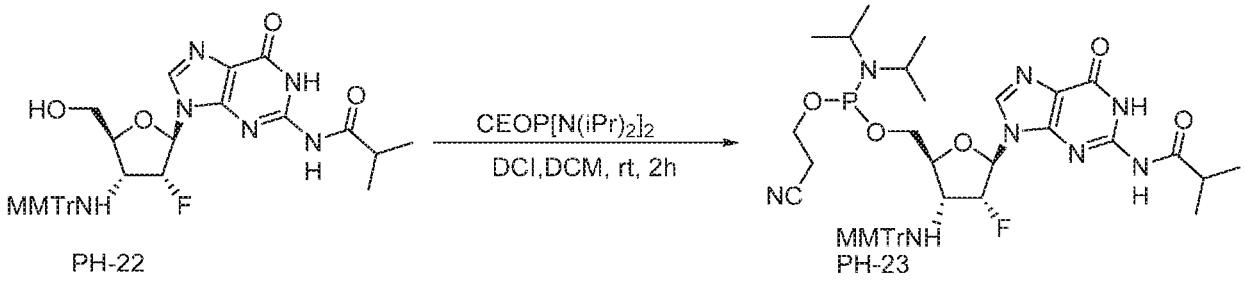


PH-22

[0238] To a solution of **PH-21** (6.0 g, 16.95 mmol, 1.00equiv) in pyridine/N,N-diisopropylethylamine (100/20 mL) with an inert atmosphere of argon, was added 1-(chlorodiphenylmethyl)-4-methoxybenzene (6.24 g, 20.34 mmol, 1.20equiv). The resulting solution was stirred for 16 h at room temperature. The resulting solution was diluted with 1000 ml of dichloromethane. The resulting solution was washed with 1x250 mL of saturated sodium bicarbonate, 1x250 ml of water and 1x250 mL of saturated sodium chloride respectively. The residue was applied onto a silica gel column with dichloromethane/methanol (100/1~50/1). This resulted in 13 g (74%) of **PH-22** as a white solid. 1H-NMR (DMSO-d_6, 400MHz): 12.15 (s, 1H), 11.70 (s, 1H), 8.14 (s, 1H), 7.49 (m, 4H), 7.24 (m, 6H), 7.15 (m, 2H), 6.72 (m, 2H), 5.82

(m, 1H), 5.30 (m, 1H), 4.04 (m, 3H), 3.62 (s, 3H), 3.45 (m, 1H), 2.83-2.62 (m, 3H), 1.10 (m, 6H).

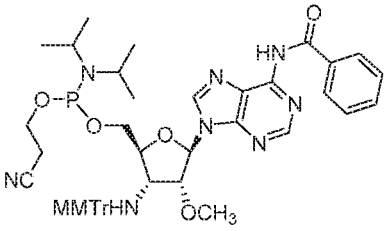
Preparation of PH-23



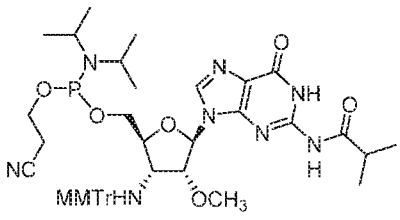
[0239] To a solution of **PH-22** (7.8 g, 12.45 mmol, 1.00 equiv.) in dichloromethane (80 mL) with an inert atmosphere of argon, was added 3-(bis[bis(propan-2-yl)amino]phosphanyloxy)propanenitrile (7.5 g, 24.92 mmol, 2.00 equiv.) and 4,5-dicyanoimidazole (2.2 g, 18.63 mmol, 1.50 equiv.) in order. The resulting solution was stirred for 2 h at room temperature. The resulting mixture was diluted with 1000 mL of dichloromethane. The resulting solution was washed with 3x250 mL of saturated sodium bicarbonate, 3x250 mL of water and 3x250 mL of saturated sodium chloride respectively. The resulting solution was concentrated under reduced pressure. The crude product was purified by Flash-Prep-HPLC with the following conditions: Column, C18 silica gel; mobile phase, waters and acetonitrile (40% acetonitrile up to 95% in 35 min); Detector, UV 254 nm. This resulted in 8.06 g (78%) of **PH-23** as a white solid. MS m/z [M+H] + (ESI): 827.

2'-F-3'-NHTr building blocks for oligomer synthesis

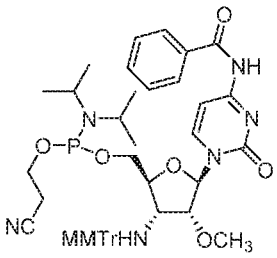
[0240] The 2'-O-Me 3'-NH-MMTr-5'-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl) phosphoramidite monomers of 6-*N*-benzoyladenosine (A^{Bz}), 4-*N*-Benzylcytidine (C^{Bz}), 2-*N*-isobutyrylguanosine (G^{iBu}), and Uridine (U) as shown below were synthesized using the procedure described in WO 200118015 A1



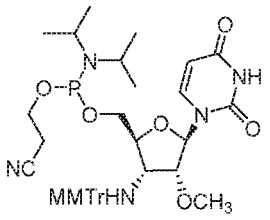
3'-NHMMTr-2'-O-Me A (Bz)



3'-NHMMTr-2'-O-Me-G(iBu)



3'-NHMMTr-2'-O-Me-C(Bz)



3'-NHMMTr-2'-OMe U:

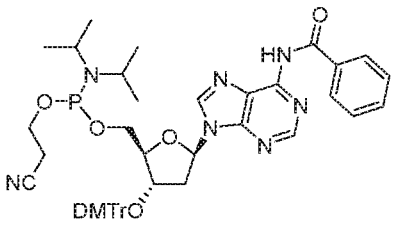
2'-O-Me-3'-NHTr building blocks for oligomer synthesis

[0241] Exemplary phosphoroamidates include:

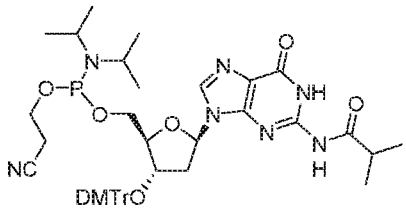
Raw material description
3'-NHTr-dA(Bz)
3'-NHTr-dC(Bz)
3'-NHTr-dG(iBu)
3'-NHTr-T:
3'-NHMMTr-2`-F-A(NH-Bz)
3'-NHMMTr-2'-F-C(NH-Bz)
3'-NHMMTr-2'-F-G(NH-iBu)
3'-NHMMTr-2'-F-U:
3'-NHMMTr-2`-OMe-A(NH-Bz)
3'-NHMMTr-2'-OMe-C(NH-Bz)
3'-NHMMTr-2'-OMe-G(NH-iBu)
3'-NHMMTr-2'-OMe U:
3'-NHTr (dA, dC, dG and dT)-CPG 500Å: Loading: 64-83 µmol/g

The reverse phosphoramidite 3'-O-DMT-deoxy Adenosine (NH-Bz), 5'-O-(2-cyanoethyl-N,N-diisopropyl phosphoramidite, 3'-O-DMT-deoxy Guanonosine (NH-ibu), 5'-O-(2-cyanoethyl-N,N-diisopropyl phosphoramidite, 3'-O-DMT-deoxy Cytosine (NH-Bz), 5'-O-(2-cyanoethyl-N,N-diisopropyl phosphoramidite, 3'-O-DMT-deoxy Thymidine (NH-Bz), 5'-O-(2-cyanoethyl-

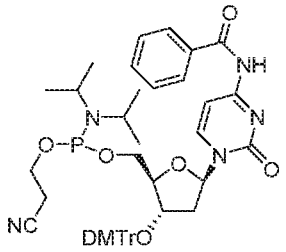
N,N-diisopropyl phosphoramidite and reverse solid supports were purchased from commercially-available sources (Chemgenes).



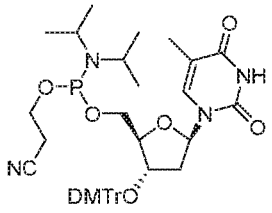
3'-DMTr-dA (Bz)



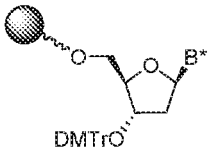
3'-DMTr-dG(iBu)



3'-DMTr-dC(Bz)



3'-DMTr-T:



B* = A, C, G or T

Reverse DNA building blocks for oligomer synthesis

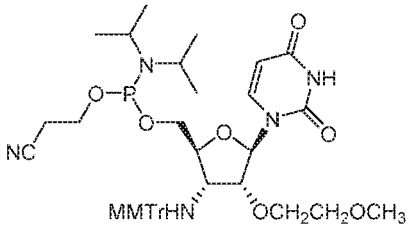
[0242] Exemplary reverse phosphoroamidites used for this disclosure include:

Raw material description
3'-O-DMTr-2'-OMe-A(NH-Bz)
3'-O-DMTr-2'-OMe-C(NH-Bz)
3'-O-DMTr-2'-OMe-G(NH-iBu)
3'-O-DMTr-2'-OMe-U:
3'-ODMTr (dA, dC, dG and dT)-CPG 500Å: Loading: 64-83 µmol/g

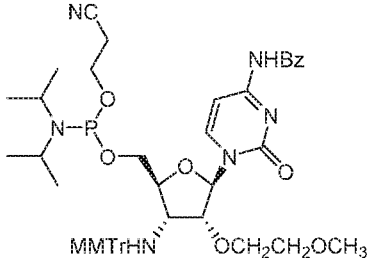
[0243] For making the oligomers with the following modifications: 2'-F-NPS-PS-2'-F-NPS ; 2'-F-NP-PS-2'-F-NP; 2'-OMe-NP-PS-2'-OMe-NP; 2'-OMe-NPS-DNA-PS-2'-OMe-NPS, the synthesis was carried out on a 1 µM scale in a 5' to 3' direction with the 5'-phosphoramidite monomers diluted to a concentration of 0.1 M in anhydrous CH_3CN in the presence of 5-(benzylthio)-1H-tetrazole activator (coupling time 2.0-4.0 min) to a solid bound oligonucleotide followed by standard capping, oxidation and deprotection afforded modified oligonucleotides. The stepwise coupling efficiency of all modified phosphoramidites was more than 98%. The

DDTT (dimethylamino-methylidene) amino)-3H-1, 2, 4-dithiazaoline-3-thione was used as the sulfur-transfer agent for the synthesis of oligoribonucleotide phosphorothioates. Oligonucleotide-bearing solid supports were heated at room temperature with aqueous ammonia/Methylamine (1:1) solution for 3 h in shaker to cleavage from support and deprotect the base labile protecting groups.

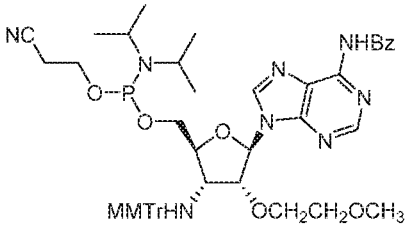
Examples 1-4



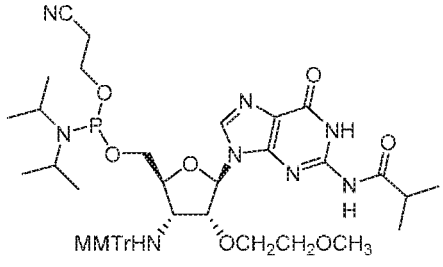
Example 1



Example 2



Example 3



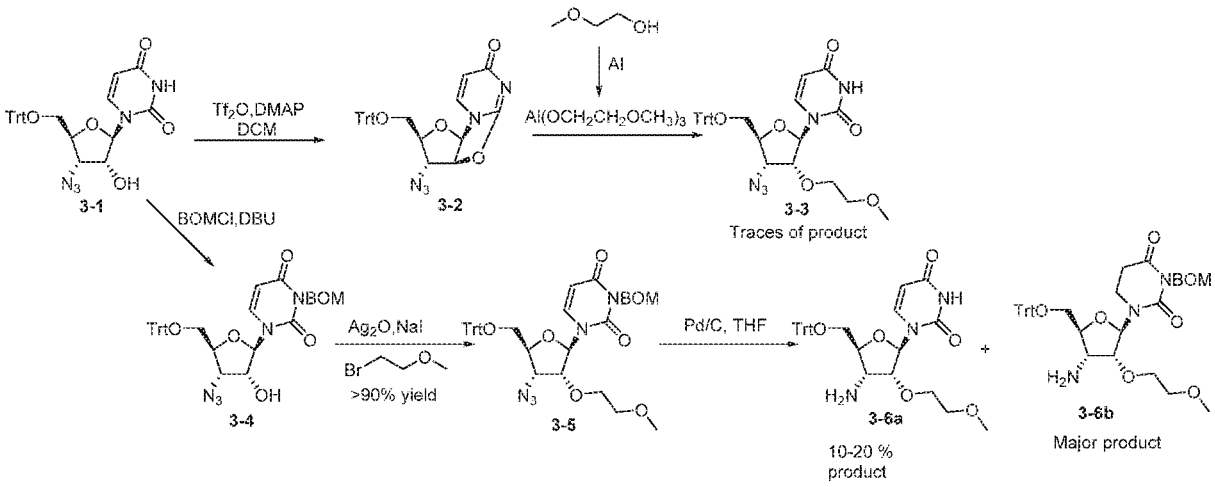
Example 4

[0244] The appropriately protected 2'-O-methoxy ethyl-3'-aminonucleoside-5'-phosphoramidite building blocks (examples 1-4 were prepared after chemical transformations shown in Schemes 1-4.

[0245] First for synthesis of uracil based 3'-NH-MMTr-2'-O-methoxyethyl phosphoramidites example 5, key 3'-azido-2'-methoxyethyl intermediate 3 was obtained in low yields via an-hydro intermediate 2 as shown in scheme 1.

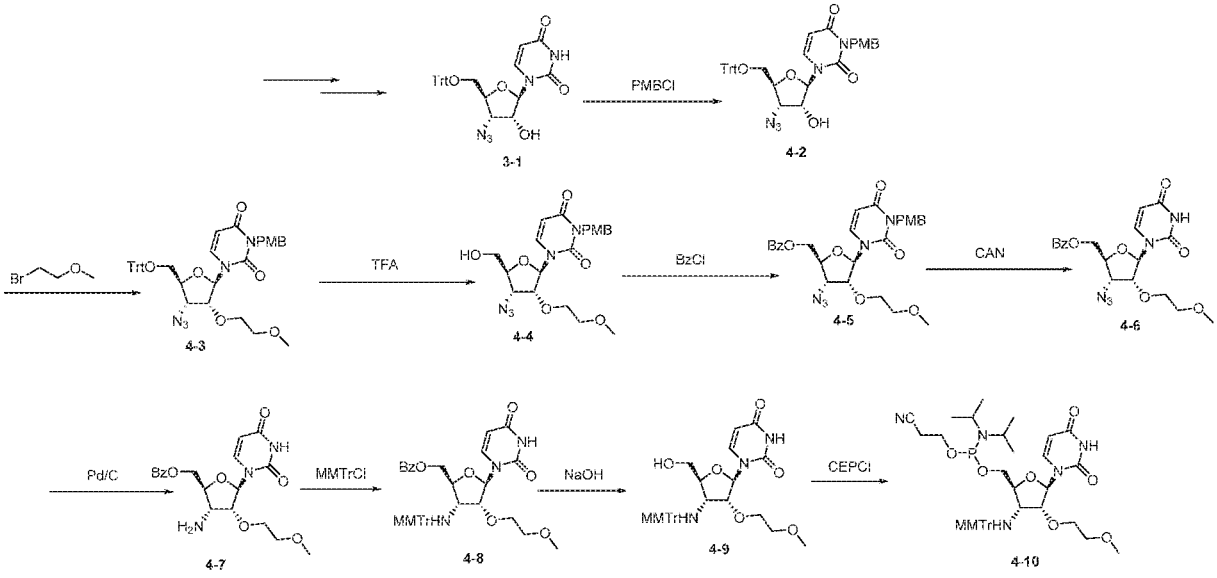
[0246] Due to low yielding alkylation, 3-1 was reacted with BOMCl/ DBU to give N-3 protected intermediate **3-4**, which was alkylated by using 2-bromoethyl methyl ether/ Ag_2O/ NaI/DMF to give 2'-O-methoxyethyl derivative 3-5 as shown below in scheme 1. Deprotection of N-3-BOM group using hydrogenation condition ($Pd/C/H_2$) resulted in 10-20% desired 3'-amino intermediate3- 6a along with significant over reduced side product 3-6b.

Scheme 1



[0247] 2'-O-alkylation in high yield is obtained as shown below in scheme 2. For this purpose, 3-1 was treated with PMBCl/ DBU/ DMF to give N-3 protected intermediate 4-2, which was subjected for 2'-O alkylation using 2-bromoethyl methyl ether/ Ag_2O/ NaI/DMF to give 2'-O-methoxyethyl derivative 4-3. Then, 5'-de-tritylation of 4-3 and re-protection of 5'- hydroxyl group using benzoyl chloride afforded 4-5.

Scheme 2



[0248] De-protection of PMB group of intermediate 4-5 in mild conditions gives 4-6. 3'-Azido group of intermediate 4-6 was reduced to an amine, which was then immediately protected, such

as reaction with 4-monomethoxytritylchloride, to give 4-8. The 5'-benzyl ester was then cleaved using an alkaline solution, followed by phosphitylation using known protocols to give the desired 2'-O-methoxyethoxy uridine phosphoramidite monomer 4-10.

[0249] Preparation of (**4-2**): To a solution of **3-1** (45.30 g, 88.56 mmol) in DMF (120.00 mL) was added PMBCl (20.80 g, 132.84 mmol) and DBU (44.61 g, 177.12 mmol), the mixture was stirred at r.t. for 2 h. Water was added, extracted with EA. The organic layer was concentrated and purified by column to give **4-2** (52.00 g, 82.32 mmol) as a white solid. ESI-LCMS: m/z 632.3 $[M+H]^+$.

[0250] Preparation of (**4-3**): To a solution of **4-2** (50.00 g, 79.15 mmol) in DMF (120.00 mL) was added 2-Bromoethyl methyl ether (16.50 g, 118.73 mmol) and Ag_2O (18.34 g, 79.15 mmol, 2.57 mL), then NaI (5.93 g, 39.58 mmol) was added. The reaction mixture was stirred at r.t. for 12 h. LC-MS showed work well. Filtered and added water and EA, the organic layer was concentrated and purified by column to give **4-3** (52.00 g, 75.39 mmol) as a colorless oil. ESI-LCMS: m/z 690.4 $[M+H]^+$.

[0251] Preparation of (**4-4**): To a solution of **4-3** (52.00 g, 75.39 mmol) in DCM (200.00 mL) was added TFA (150.00 mL). The mixture was stirred at r.t. for 1 h. The reaction mixture was slowly added to cold NH_4OH, extracted with DCM. The organic layer was concentrated and purified to give **4-4** (31.00 g, 69.28 mmol) as a colorless oil. ESI-LCMS: m/z 448.2 $[M+H]^+$. ^1H-NMR (DMSO-d_6, 400MHz): δ ppm 8.02 (d, J = 8.12Hz, 1H), 7.26-7.23 (m, 2H), 6.87-6.84 (m, 2H), 5.87-5.81 (m, 2H), 5.38 (t, J = 5.0Hz, 1H), 4.96-4.85 (m, 2H), 4.36-4.34 (m, 1H), 4.17-4.14 (m, 1H), 4.00-3.97 (m, 1H), 3.83-3.77 (m, 1H), 3.75-3.72 (m, 1H), 3.71 (s, 3H), 3.70-3.68 (m, 1H), 3.61-3.56 (m, 1H), 3.45-3.43 (m, 2H), 3.18 (s, 3H).

[0252] Preparation of (**4-5**): To a solution of **4-4** (31.00 g, 69.28 mmol) in Pyridine (200.00 mL) was added BzCl (13.14 g, 93.87 mmol), the reaction mixture was stirred at r.t. for 15 min and concentrated and purified by column to give **4-5** (35.10 g, 63.8 mmol) as a white solid. ESI-LCMS: m/z 552.2 $[M+H]^+$.

[0253] Preparation of (**4-6**): To a solution of **4-5** (35.10 g, 63.8 mmol) in acetonitrile (300.00 mL) and water (100.00 mL) was added Ceric ammonium nitrate (105 g, 191.40 mmol), the reaction mixture was stirred at r.t. for 12 h and concentrated and extracted with EA. The organic

layer was concentrated and purified by column to give **4-6** (27.5 g, 63.75 mmol) as a yellow solid. ESI-LCMS: m/z 432.2 $[M+H]^+$.

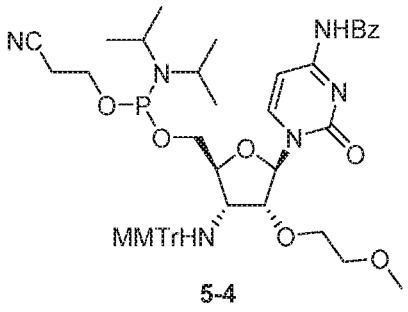
[0254] Preparation of (**4-7**): To a solution of **4-6** (27.50 g, 63.75 mmol) in THF (500.00 mL) was added Pd/C (3.00 g), the reaction mixture was stirred at r.t. for 12 h and filtered and concentrated to give **4-7** (25.00 g, 61.67 mmol) as a yellow solid. ESI-LCMS: m/z 406.2 $[M+H]^+$.

[0255] Preparation of (**4-8**): To a solution of **4-7** (25.00 g, 61.67 mmol) in DCM (300.00 mL) was added MMTrCl (28.49 g, 92.51 mmol) and Collidine (14.95 g, 123.34 mmol), then$AgNO_3$ (15.7 g, 92.5 mmol) was added. The reaction mixture was stirred at r.t. for 1h., and filtered and the organic layer was washed water, dried over Na_2SO_4 and purified by silica gel column to give **4-8** (33.00 g, 48.69 mmol) as a yellow solid.

[0256] Preparation of (**4-9**): To a solution of **4-8** (14.50 g, 21.39 mmol) was added 1 N NaOH in methanol (200 mL) in water (20 mL), the reaction mixture was stirred at r.t. for 1 h. and concentrated and extracted with DCM, the organic layer was concentrated and purified by silica gel column to give **4-9** (11.50 g, 20.05 mmol) as a white solid. ^{1}H-NMR (DMSO-d_6, 400MHz): δ ppm 11.26 (s, 1H), 7.95 (d, J = 8.4Hz, 1H), 7.47-7.44 (m, 4H), 7.34-7.17 (m, 8H), 6.82 (d, J = 8.8Hz, 2H), 5.50-5.48 (m, 2H), 5.13 (t, J = 3.6Hz, 1H), 4.05-3.98 (m, 3H), 3.78 (s, 3H), 3.52-3.49 (m, 1H), 3.34-3.32 (m, 2H), 3.14 (s, 3H), 3.08-3.04 (m, 1H), 2.89-2.86 (m, 1H), 2.70 (d, J = 10.0 Hz, 1H), 1.51 (d, J = 4.4Hz, 1H).

[0257] Preparation of (**4-10**): To a solution of **4-9** (11.50 g, 20.05 mmol) in DCM (100.00 mL) was added DMAP (489.85 mg, 4.01 mmol) and DIPEA (10.36 g, 80.19 mmol, 14.01 mL). Then CEPCl (5.70 g, 24.06 mmol) was added to the solution. The mixture was stirred at r.t. for 30 min. The reaction was quenched with saturated $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4, concentrated to give the crude product. The crude product was purified by Flash-Prep-HPLC. The product was dissolved in anhydrous toluene and concentrated for three times. Then the product was dissolved anhydrous acetonitrile and concentrated for three times. This resulted in 13 g to give **4-10** as a white solid. MS m/z $[M-H]^-$ (ESI): 772.3; ^{1}H-NMR ($CDCl_3$, 400MHz): 9.01(s, 1H), 8.07-7.61(m, 1H), 7.53-7.41(m, 6H), 7.29-7.15 (m, 5H), 6.79-6.76 (m, 2H), 5.63-5.57 (m, 2H), 4.27-4.15 (m, 2H), 4.06-3.95 (m, 1H), 3.85-3.77(m, 1H), 3.75(s, 3H), 3.69-3.35(m, 7H), 3.23(d, J=4Hz, 1H), 2.26-2.91(m, 3H), 2.59(t, J = 6.4Hz, 1H), 1.75-1.39(m, 1H), 1.21-1.11(m, 12H). ^{31}PNMR (162 MHz, $CDCl_3$): 149.10, 148.26.

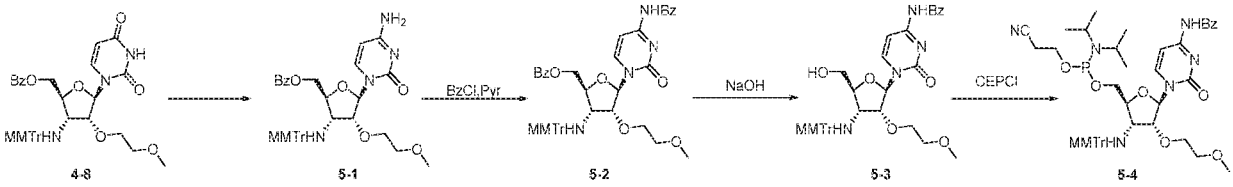
Example 5



5-4

[0258] The 2'-O-methoxyethoxy-NH-benzoyl- cytosine phosphoramidite compound **5-4** was obtained by conversion of uridine intermediate 4-8 into 3'-amino cytidine analogue 5-1 followed by phosphitylation using known protocols to give the desired 2'-O-methoxyethoxy cytidine phosphoramidite monomer 5-4 as shown below in scheme 3.

Scheme 3



4-8 5-1 5-2 5-3 5-4

[0259] Preparation of (**5-1**): To a solution of **4-8** (18.50 g, 27.30 mmol) in acetonitrile (250.00 mL) was added TPSCl (16.49 g, 54.60 mmol) and DMAP (6.67 g, 54.60 mmol), then TEA (5.52 g, 54.60 mmol, 7.56 mL) was added to the solution. The reaction mixture was stirred at r.t. for 5 h under N_2. NH_4OH (50.00 mL) was added to the reaction mixture. The mixture was stirred at r.t. for 12 h. The solution was concentrated and extracted with EA. The organic layer was washed by brine and dried over Na_2SO_4. The organic layer was concentrated and purified by silica gel column to give **5-1** (16.00 g, 23.64 mmol) as a yellow solid.

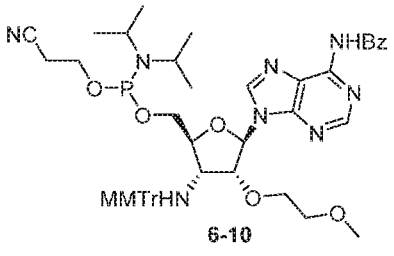
[0260] Preparation of (**5-2**): To a solution of **5-1** (16.00 g, 23.64 mmol) in Pyridine (100.00 mL) was added BzCl (4.96 g, 35.46 mmol) at 0°C. The mixture was stirred at r.t. for 1 h. The solution was concentrated and purified by silica gel column to give **5-2** (17.40 g, 22.28 mmol) as a white solid.

[0261] Preparation of (**5-3**): Compound **5-2** (17.40 g, 22.28 mmol) was added to 180 mL of 1 N NaOH solution in Pyridine/MeOH/H_2O (65/30/5) at 0 °C. The suspension was stirred at 0 °C for

15 min. The reaction mixture was quenched by addition of sat. NH_4Cl solution. The solution was extracted with EA and the combined organic layers were washed with sat. $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column to give **5-3** (12.50 g, 18.47 mmol) as white solid. 1H-NMR (DMSO-d_6, 400MHz): δ ppm 12.25 (s, 1H), 8.53 (d, J = 7.6Hz, 1H), 8.01 (d, J = 5.2Hz, 2H), 7.64-7.60 (m, 1H), 7.52-7.42 (m, 6H), 7.31 (d, J = 8.8Hz, 2H), 7.26-7.14 (m, 7H), 6.79 (d, J = 8.8Hz, 2H), 5.55 (s, 1H), 5.23 (t, J = 3.6Hz, 1H), 4.09-3.97 (m, 3H), 3.73 (s, 3H), 3.70-3.66 (m, 1H), 3.38-3.34 (m, 2H), 3.17 (s, 3H), 3.11-3.05 (m, 1H), 2.96-2.91 (m, 1H), 2.68 (d, J=10.8Hz, 1H), 1.49 (d, J=4Hz, 1H).

[0262] Preparation of (**5-4**): To a solution of **5-3** (12.50 g, 18.47 mmol) in DCM (100.00 mL) was added DMAP (451.30 mg, 3.69 mmol) and DIPEA (9.55 g, 73.88 mmol, 12.90 mL), then CEPCl (5.25 g, 22.16 mmol) was added. The mixture was stirred at r.t. for 30 min. The reaction was quenched with saturated $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4, concentrated to give the crude product. The crude was by Flash-Prep-HPLC. The product was dissolved in anhydrous toluene and concentrated for three times. Then the product was dissolved anhydrous acetonitrile and concentrated for three times. This resulted in 13 g to give **5-4** as a white solid. MS m/z $[M-H]^-$ (ESI): 875.4. ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 8.64-8.20 (m, 2H), 7.90-7.88 (m, 2H), 7.62-7.58 (m, 1H), 7.53-7.39 (m, 8H), 7.25-7.15 (m, 6H), 6.78-6.74 (m, 2H), 5.69 (d, J=1.72Hz, 1H), 4.37-4.21 (m, 2H), 4.10-4.03 (m, 1H), 3.90-3.79 (m, 2H), 3.75 (d, J=1.64Hz, 3H), 3.68-3.52 (m, 3H), 3.46-3.42 (m, 2H), 3.26 (d, J=1.2Hz, 3H), 3.17-2.97 (m, 2H), 2.94-2.87 (m, 1H), 2.67-2.48 (m, 2H), 1.79-1.51(m, 1H),1.26-1.18 (m, 12H). ^{31}PNMR (162 MHz, $CDCl_3$): 148.93, 148.03

Example 6

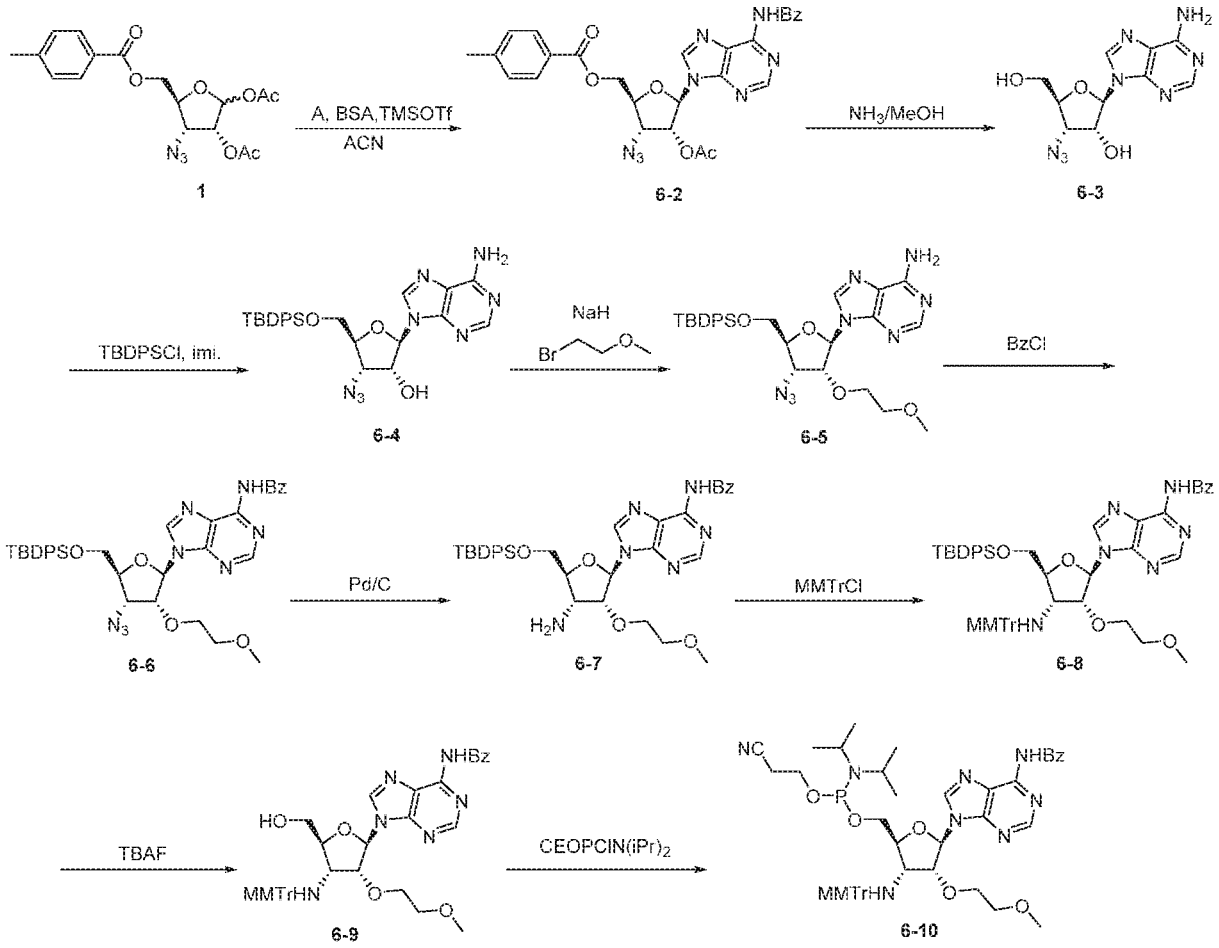


6-10

[0263] The synthesis of the 2'-O-methoxyethyl adenosine analogue **6-10** was achieved as shown below in scheme 6. The intermediate 6-2 under basic condition (NH_3/MeOH) resulted in diol 6-3, which then upon protection of 5'-hydroxy group using TBDPSCl to give 6-4 Intermediate 6-4.

Then, 2'-O alkylation of 6-4 using 2-bromoethyl methyl ether/NaH/DMF to give 2'-O-methoxyethyl derivative 6-5 without the protection of C-6-exocyclic amine of 6-4. In an inventive way selective alkylation of 2'-OH group of intermediate 6-4 was achieved.

Scheme 4



[0264] 3'-Azido group of intermediate 6-5 was reduced to the amine 6-7, which was then immediately protected, such as reaction with 4-monomethoxytritylchloride, to give the precursor 6-8 after de-protection of 5'-OTBDPS group using TBAF/THF. The phosphitylation of 6-9 using known protocols is performed to give the desired 2'-O-methoxyethoxy adenine-NH-benzoyl phosphoramidite monomer **6-10.**

[0265] Preparation of (6-2): To a solution of compound **1** (79.50 g, 210.68 mmol) in dry ACN (1.20 L) was added N-(5H-Purin-6-yl)benzamide (100.80 g, 421.36 mmol) and BSA (180.07 g,

884.86 mmol). The resulting suspension was stirred at 50°C until clear. Then the mixture was cooled at -20°C and TMSOTf (93.54 g, 421.36 mmol) was added by syringe. Then the mixture was stirred at 70°C for 72 h under N_2, and quenched with sat $NaHCO_3$ and extracted with DCM. The organic layer was dried over Na_2SO_4, then solvent was evaporated, and the residue was purified on silica gel to afford compound **6-2** (107.50 g, 192.26 mmol, 91.26% yield) as a yellow solid. ^{1}H-NMR (400 MHz, DMSO): δ = 11.28 (s, 1H), 8.64 (d, *J* = 6.4 Hz, 2H), 8.05 (d, *J* = 8.0 Hz, 2H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.66 (t, *J* = 7.6 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H), 6.37 (d, *J* = 3.6 Hz, 1H), 6.17 (dd, *J* = 6.0 Hz, 1H), 5.09 (t, *J* = 6.8 Hz, 1H), 4.69-4.56 (m, 2H), 4.40-4.38 (m, 1H), 2.39 (s, 3H), 2.17 (s, 3H). ESI-LCMS: m/z 557.2 $[M+H]^+$.

[0266] Preparation of (6-3): To a solution of compound **6-2** (107.50 g, 192.26 mmol) dissolved in 33 wt.% methylamine in ethanol (600.00 mL), then the mixture were stirred at 20°C for 16 h, then solvent was evaporated, washed with 50% EtOAc in petroleum ether (1.5 L), filtered to afford compound **6-3** (52.50 g, 179.64 mmol, 93.44% yield) as a slightly yellow solid. ESI-LCMS: m/z 293.1 $[M+H]^+$.

[0267] Preparation of (6-4): A solution of compound **6-3** (52.50 g, 179.64 mmol), imidazole (18.32 g, 269.46 mmol) and TBDPS-Cl (54.34 g, 197.60 mmol) in pyridine (500.00 mL) was stirred at 20°C for 2 h, LC-MS showed **6-3** was consumed. Then quenched with MeOH (30 mL), concentrated to give the crude product which was purified on silica gel with to afford compound **6-4** (72.60 g, 136.81 mmol, 76.16% yield) as a white solid. ^{1}H-NMR (400 MHz, DMSO): δ = 8.29 (s, 1H), 8.10 (s, 1H), 7.63-7.59 (m, 4H), 7.48-7.33 (m, 8H), 6.36 (d, *J* = 5.6 Hz, 1H), 5.97 (d, *J* = 4.4 Hz, 1H), 5.10-5.06 (m, 1H), 4.47 (t, *J* = 5.6 Hz, 1H), 4.14-4.11 (m, 1H), 3.94 (dd, *J* = 11.2 Hz, 1H), 3.83 (dd, *J* = 11.6 Hz, 1H), 0.99 (s, 9H). ESI-LCMS: m/z 531.3 $[M+H]^+$.

[0268] Preparation of (6-5): A solution of **6-4** (35.00 g, 65.96 mmol) and 1-Bromo-2-methoxyethane (18.33 g, 131.91 mmol) in dry DMF (400.00 mL), was added NaI (19.77 g, 131.91 mmol) and Ag_2O (15.29 g, 65.96 mmol), the mixture was stirred at room temperature for 5 h. Then the reaction was poured into ice water, extracted with EA, washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated, and the residue was purified on silica gel to give **6-5** (23.70 g, 40.26 mmol, 61.04% yield) as a white solid and by-product of TBDPS lost 5.20 g, 9.81 mmol, 14.87% yield) as a white solid. ^{1}H-NMR (400 MHz, DMSO): δ = 8.31 (s, 1H), 8.11 (s, 1H), 7.63-7.60 (m, 4H), 7.47-7.44 (m, 2H), 7.40-7.36 (m, 6H), 6.10 (d, *J* = 4.4 Hz,

1H), 5.02 (t, J = 4.8 Hz, 1H), 4.69 (t, J = 5.6 Hz, 1H), 4.18-4.14 (m, 1H), 3.95 (dd, J = 11.6 Hz, 1H), 3.84 (dd, J = 11.6 Hz, 1H), 3.78-3.75 (m, 2H), 3.45 (t, J = 4.8 Hz, 1H), 3.16 (s, 3H), 0.99 (s, 9H). ESI-LCMS: m/z 589.5 $[M+H]^+$.

[0269] Preparation of (6-6): To a solution of **6-5** (31.23 g, 53.04 mmol) in pyridine (300.00 mL) at 0°C, was added BzCl (11.22 g, 79.56 mmol) dropwise. The mixture was stirred at r.t. for 2 h. Then the solution was cooled to 0°C, and ammonium hydroxide (20 mL, 30%) was added and the mixture was allowed to warm to r.t., then the solvent was evaporated, 300 mL H_2O and 600 mL EA were added into separate the solution, the aqueous was extracted by EA, combined the organic and washed with brine, dried over anhydrous Na_2SO_4, the solvent was removed and the residue was purified on silica gel to give **6-6** (28.70 g, 41.42 mmol, 78.09% yield) as a white solid. ESI-LCMS: m/z 693.4 $[M+H]^+$.

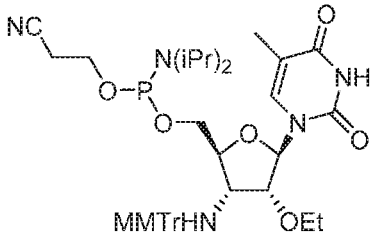
[0270] Preparation of (6-7): A solution of **6-6** (28.70 g, 41.42 mmol) in EA (150.00 mL) was added Pd/C (3.00 g) and MeOH (150.00 mL) under H_2. The mixture was stirred at r.t. for 5 h. Then the reaction was filtered and the filtrate concentrated to give **6-7** (25.49 g, 38.22 mmol, 92.27% yield) as a gray solid. ESI-LCMS: m/z 667.3 $[M+H]^+$.

[0271] Preparation of (6-8): To a solution of **6-7** (25.49 g, 38.22 mmol) and $AgNO_3$ (12.98 g, 76.44 mmol) in DCM (300.00 mL) was added collidine (13.89 g, 114.66 mmol) and MMTrCl (19.43 g, 57.33 mmol), the mixture was stirred at r.t. for 2 h. Then the reaction was poured into ice water, the organic layer extracted with DCM, washed with brine and dried over anhydrous Na_2SO_4, the solvent was removed and the residue was purified on silica gel to give **6-8** (32.79 g, 34.92 mmol, 91.36% yield) as a gray solid.

[0272] Preparation of (6-9): A solution of **6-8** (32.79 g, 34.92 mmol) in THF (300.00 mL) was added TBAF (1M, 35.00 mL), the mixture was stirred at room temperature for 15 h. Then the solvent was removed and the residue was purified on silica gel with EA to give **6-9** (22.22 g, 31.71 mmol, 90.82% yield) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.68 (s, 1H), 8.32 (s, 1H), 8.04 (d, J = 7.2 Hz, 2H), 7.61-7.57 (m, 1H), 7.53-7.48 (m, 6H), 7.40 (d, J = 8.8 Hz, 2H), 7.21-7.12 (m, 6H), 6.73 (d, J = 8.8 Hz, 2H), 6.09 (d, J = 2.4 Hz, 2H), 4.08-4.02 (m, 2H), 3.93-3.87 (m, 1H), 3.72 (s, 3H), 3.58-3.53 (m, 1H), 3.43-3.39 (m, 3H), 3.24-3.19 (m, 4H), 2.19 (br, 1H).

[0273] Preparation of (6-10): To a solution of **6-9** (14.00 g, 19.98 mmol), DMAP (488.19 mg, 4.00 mmol) and DIPEA (6.46 g, 49.95 mmol, 8.73 mL) in dry DCM (100.00 mL) was added CEPCl (5.68 g, 23.98 mmol) dropwise under Ar. The mixture was stirred at room temperature for 1 h. Then the reaction was wished with 10% $NaHCO_3$ (aq) and brine, dried over Na_2SO_4, the solvent was removed and the residue was purified by c.c. with the PE/EA mixture, then concentrated to give the crude product. The crude product (10 g, dissolved in 10 mL of ACN) was purified by Flash-Prep-HPLC to obtain **6-10** (12.60 g, 13.98 mmol, 69.99% yield) as a white solid. Then the product was dissolved in dry toluene (15 mL) and concentrated three times, and with dry ACN three times. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.12 (d, J = 46.8 Hz, 1H), δ = 8.71 (d, J = 11.6 Hz, 1H), 8.50 (s, 0.6H), 8.22 (s, 0.4H), 8.04 (t, J = 7.2 Hz, 2H), 7.63-7.59 (m, 1H), 7.55-7.46 (m, 6H), 7.40-7.37 (m, 2H), 7.19-7.06 (m, 6H), 6.69 (dd, J = 8.8 Hz, 2H), 6.03 (d, J = 3.2 Hz, 1H), 4.36-4.24 (m, 2H), 3.92-3.78 (m, 2H), 3.71 (d, J = 11.6 Hz, 3H), 3.67-3.33 (m, 7H), 3.29 (d, J = 11.2 Hz, 3H), 3.17-3.10 (m, 1H), 2.88 (dd, J = 27.2 Hz, 1H), 2.65-2.50 (m, 2H), 2.38 (d, J = 4.4 Hz, 0.4H), 1.80 (d, J = 4.0 Hz, 0.6H), 1.23-1.15 (m, 12H). ^{31}PNMR (400 MHz, $CDCl_3$): 148.86, 148.22. ESI-LCMS: m/z 901.3 $[M+H]^+$.

Example 7

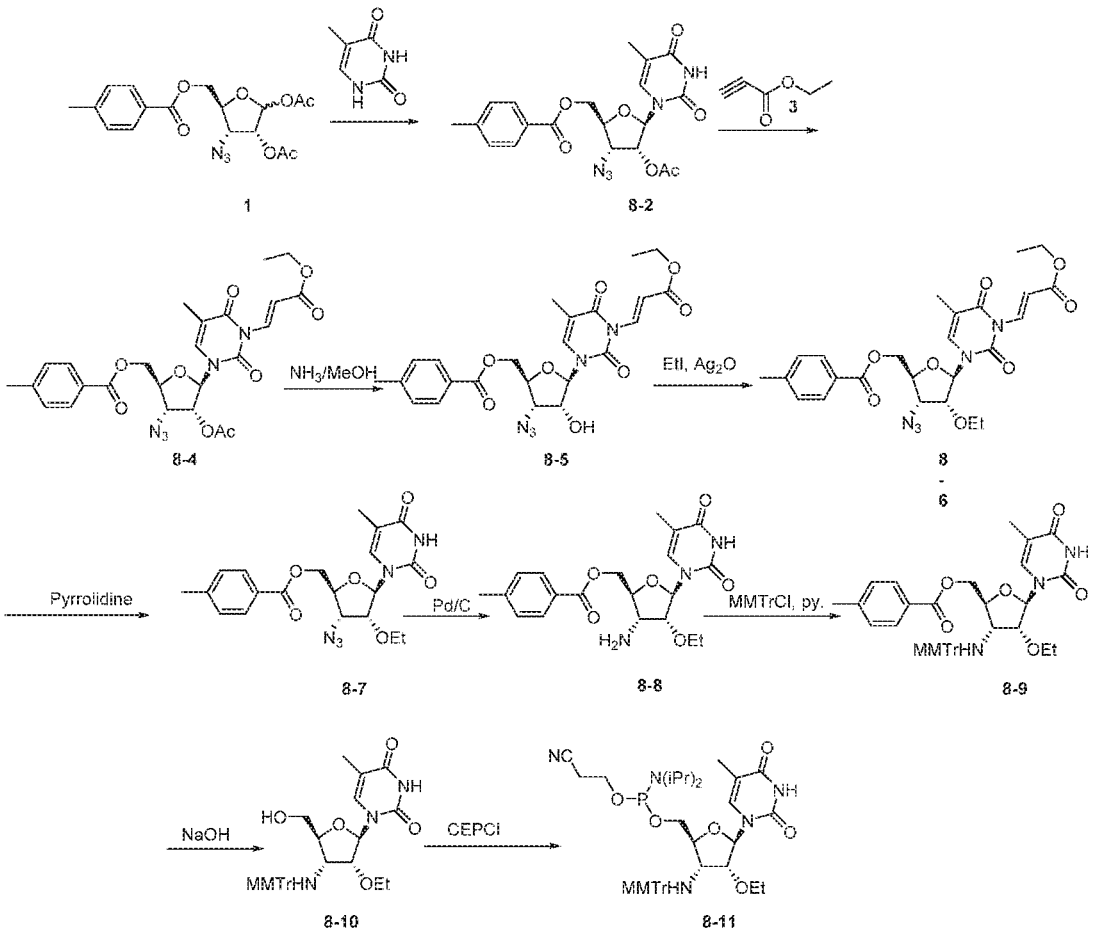


8-11

[0274] The appropriately protected 2'-O-ethyl-3'-amino-5'-phosphoramidite (example 9, 10, 11, 12), were prepared after chemical transformations shown in Schemes 8-12.

[0275] First for the synthesis of thymine based 3'-NH-MMtr-2'-O-ethyl phosphoramidites example 9, intermediate 2 was protected such as methyl propyolate in the presence of dimethylaminopyridine (Scheme 8) to give base N-3 protected intermediate **8-4** to facilitate the 2'-O-alkylation in higher yield. Further deacetylation of **8-4** to give C-2'-hydroxy intermediate 8-5.

Scheme 5



[0276] Further alkylation using iodoethane afforded 2'O-ethyl nucleoside **8-6**. Intermediate **8-6** was converted to thymine base 2'-O-ethyl-3'-amino-5'-phosphoramidite **8-11** by following the similar chemistry for compound **4-10** shown in previous Scheme 4.

[0277] Preparation of (8-4): To a solution of 8-2 (22.0 g, 49.62 mmol) in MeCN (400 mL) was added DMAP (1.2 g, 9.92 mmol). Then 3 (5.8 g, 419.5 mmol) was added, the mixture was stirred at r.t. for 2 h under N_2, TLC showed 8-2 was consumed. Concentrated and purified by a silica gel column by (PE:EA = 6:1) to afford 8-4 (22.0 g, 40.63 mmol, 81.9% yield) as a yellow oil. ESI-LCMS: m/z 564 $[M+Na]^+$.

[0278] Preparation of (**8-5**): To a solution of **8-4** (28.0 g, 51.71 mmol) in MeOH (400 mL) was added con. NH_4OH aqueous solution (28 mL) at 0°C. The reaction mixture was stirred at 0°C for 1.5 h, TLC showed **8-4** was consumed. Concentrated and purified by a silica gel column by

(PE:EA = 10:1~2:1) to afford **8-5** (21.0 g, 42.04 mmol, 81.3% yield) as a yellow oil. ESI-LCMS: m/z 522 $[M+Na]^+$.

[0279] Preparation of (**8-6**): To a solution of **8-5** (20.0 g, 40.04 mmol) in iodoethane (100 mL) was added Ag_2O (18.6 g, 80.08 mmol,). The reaction mixture was stirred at 50°C for 5 h, after LC-MS show totally consumed of **8-5** filtered with diatomite and concentrated to afford **8-6** (16.0, 30.33 mmol, 75.7% yield) as a yellow oil which was used directly in next step. ESI-LCMS: m/z 528 $[M+H]^+$.

[0280] Preparation of (**8-7**): To a solution of **8-6** (16.0 g, 30.33 mmol) in MeCN (400 mL) was added pyrrolidine (8.63 g, 121.32 mol, 12 mL) , the reaction mixture was stirred at r.t. overnight, TLC showed **8-6** was totally consumed. Concentrated and purified by a silica gel column by (DCM:MeOH = 100:1~50:1) to afford 7 (12.0 g, 27.94 mmol, 92.1% yield) as a yellow oil. ESI-LCMS: m/z 430 $[M+H]^+$.

Preparation of (**8-8**): To a solution of **8-7** (12.0 g, 27.94 mmol) in THF (200 mL) was added Pd/C (1.2 g), the mixture was stirred at r.t. under H_2 overnight. LC-MS showed 7 was totally consumed. Filtered and washed with DCM (100 mL * 3), then concentrated to afford **8-8** (11.0 g, 27.27 mmol, 97.6% yield) as a gray solid which was used directly in next step. ESI-LCMS: m/z 404 $[M+H]^+$.

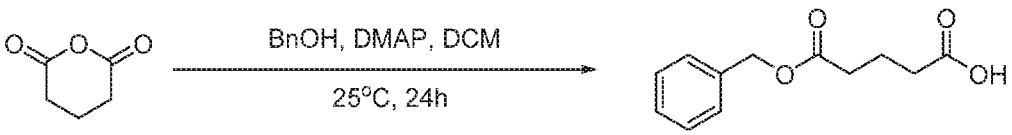
[0281] Preparation of (**8-9**): To a solution of **8-8** (10.0 g, 24.79 mmol) in DCM (80 mL) was added MMTrCl (11.4 g, 37.18 mmol), 2,4,6-collidine (2.0 g, 16.61 mmol, 6.5 mL) and $AgNO_3$ (6.3 g, 37.18 mmol), the mixture was stirred at r.t. for 1.5 h. TLC showed **8-8** was totally consumed. Filtered and the organic layer was washed with water and dried over Na_2SO_4, then concentrated and purified by a silica gel column by (PE:EA=5:1~1:1) to afford **8-9** (16.0 g, 23.68 mmol, 95.5% yield) as a light-yellow solid.

[0282] Preparation of (**8-10**): 8-9 (4.0 g, 5.92 mmol) was added to the solution of 1.0 N NaOH solution (20 mL, $MeOH/H_2O$ = 9:1). The reaction mixture was stirred at 40°C for 2 h, TLC showed **8-9** was consumed, concentrated and extracted with DCM (20 mL * 2), the organic layer was dried over Na_2SO_4 and concentrated, the residue was purified by a silica gel column by (DCM:MeOH=200:1~50:1) to afford **8-10** (3.0 g, 53.8 mmol, 90.9 yield) as a white solid.

[0283] Preparation of (**8-11**): To a solution of **8-10** (2.36 g, 4.23 mmol) in DCM (2.0 mL) was added DMAP (103 mg, 0.8 mmol) and DIPEA (2.2 g, 16.92 mmol, 2.96 mL). Then CEPCl (1.0 g, 4.23 mmol) was added. The reaction mixture was stirred at r.t. for 1 h. TLC showed **8-10** was consumed, washed with saturated $NaHCO_3$ (5 mL), separated the organic layer and washed the water layer with DCM (10 mL * 2). The combined organic layer was washed with brine, dried over Na_2SO_4, concentrated, and purified by Flash-Prep-HPLC to afford **8-11** (2.45 g, 3.23 mmol, 76.36% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.74 (dd, J = 1.4 Hz, 0.5H), 7.60-7.50 (m, 4H), 7.51-7.41 (m, 2H), 7.34- 7.16 (m, 7H), 7.12 (d, *J* = 1.4 Hz, 0.5H), 6.88-6.76 (m, 2H), 5.66 (s, 1H), 4.37-4.23 (m, 1H), 4.16-4.05 (m, 1H), 4.05-3.94 (m, 0.5H), 3.88-3.74 (m, 4.5H), 3.72-3.35 (m, 3H), 3.22 (td, *J* = 10.3, 4.7 Hz, 0.5H), 3.03-2.89 (m, 1.5H), 2.80-2.69 (m, 1H), 2.61 (t, *J* = 6.5 Hz, 1H), 2.37 (td, *J* = 6.6, 1.3 Hz, 1H), 1.97 (d, *J* = 3.5 Hz, 0.5H), 1.91 (dd, *J* = 11.4, 1.2 Hz, 3H), 1.52 (d, *J* = 4.7 Hz, 0.5H), 1.29-1.17 (m, 12H), 1.08 (td, *J* = 7.0, 4.9 Hz, 3H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.31, 147.14. ESI-LCMS: m/z 576 $[M+H]^+$.

GalNAc Synthesis

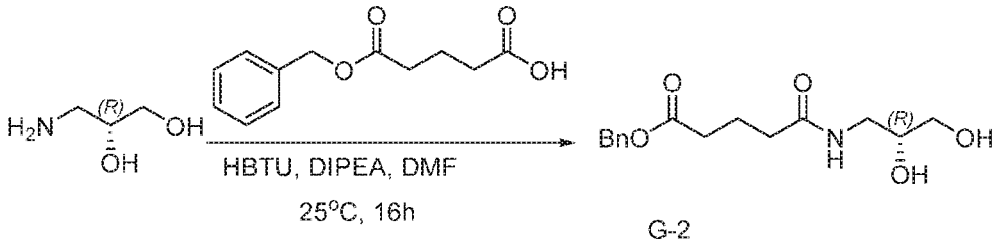
Synthesis of G-1



G-1

[0284] To a solution of oxane-2, 6-dione (1000 g, 8.76 mol, 1.00 equiv.), 4-dimethylaminopyridine (53.5 g, 437.9 mmol, 0.05 equiv.) in dichloromethane (10000 mL) with an inert atmosphere of nitrogen was added phenylmethanol (900 g, 8.32 mol, 0.95 equiv.) dropwise with stirring at room temperature. The resulting solution was stirred overnight at room temperature. The resulting mixture was washed with saturated sodium bicarbonate solution. The pH value of the aqueous layers was adjusted to 1 with 10% hydrochloric acid. The resulting solution was extracted with 3x2000 mL of ethyl acetate and the organic layers combined. The resulting mixture was washed with 2x3000 mL of saturated sodium chloride. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. This resulted in 1240 g (64%) of **G-1** as colorless oil. MS m/z [M+H]+ (ESI): 223.

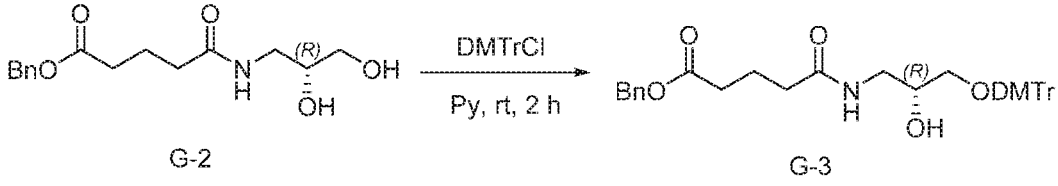
Synthesis of G-2



G-2

[0285] To a solution of **G-1** (58.5 g, 263.23 mmol, 1.20 equiv.), N, N-diisopropylethylamine (34 g, 263.57 mmol, 1.20 equiv.) in N, N-dimethylformamide (600 mL) with an inert atmosphere of nitrogen was added O-Benzotriazole-N, N, N', N'-tetramethyl-uronium-hexafluorophosphate (100 g, 263.69 mmol, 1.20 equiv.) at room temperature. The resulting solution was stirred for 1 h at room temperature. This was followed addition of (2R)-3-aminopropane-1, 2-diol (20 g, 219.52 mmol, 1.00 equiv.) at room temperature. The resulting solution was allowed to react, with stirring, overnight at room temperature. The resulting solution was diluted with 2000 mL of ethyl acetate. The resulting mixture was washed with 2x1000 mL of saturated sodium bicarbonate solution. The mixture was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was applied onto a silica gel column with dichloromethane/methanol (1:100-1:10). This resulted in 38.7 g (60%) of **G-2** as a light yellow solid. MS m/z [M+H]+ (ESI): 296.

Synthesis of G-3

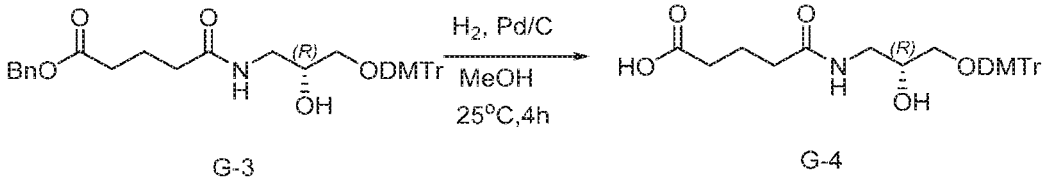


G-2 G-3

[0286] To a solution of **G-2** (10 g, 33.86 mmol, 1.00 equiv.) in pyridine (100 mL) with an inert atmosphere of nitrogen was added 1-[chloro(4-methoxyphenyl)benzyl]-4-methoxybenzene (12.63 g, 37.28 mmol, 1.10 equiv.) at room temperature. The resulting solution was stirred overnight at room temperature. The reaction was then quenched by the addition of methanol (10 mL). The resulting mixture was concentrated under reduced pressure. The resulting solution was diluted with 1000 mL of ethyl acetate. The resulting mixture was washed with 2x500 mL of saturated sodium bicarbonate solution. The mixture was dried over anhydrous sodium sulfate and

concentrated under reduced pressure. The residue was applied onto a silica gel column with dichloromethane/methanol (1:100-1:50). This resulted in 10.2 g (50%) of **G-3** as light yellow oil. MS m/z [M+Na]+ (ESI): 620.

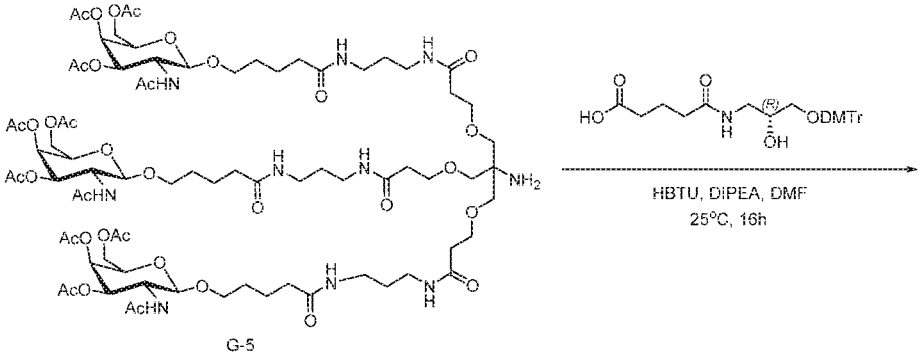
Synthesis of G-4



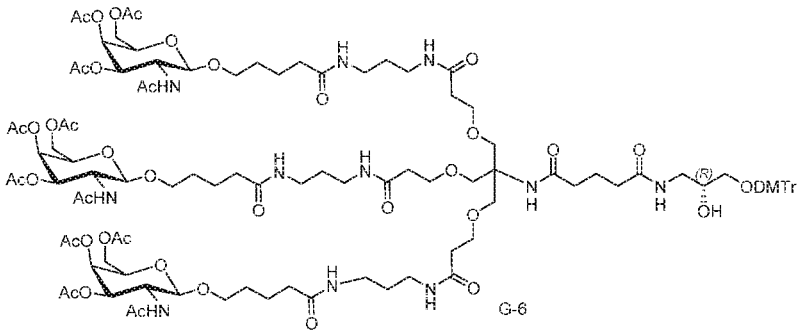
G-3 G-4

[0287] To a solution of **G-3** (10 g, 16.73 mmol, 1.00 equiv.) in methanol (100 mL) was added 10% Palladium on activated carbon (1 g) at room temperature. The flask was evacuated and flushed five times with hydrogen. The resulting solution was stirred for 4 h at room temperature. The solids were filtered out. The resulting mixture was concentrated under reduced pressure. This resulted in 7.6 g (89%) of **G-4** as a white solid. MS m/z [M+Na]+ (ESI): 530.

Synthesis of G-5



G-5

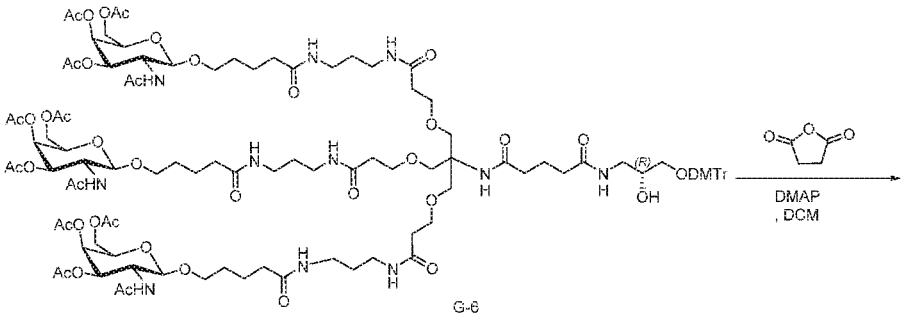


G-6

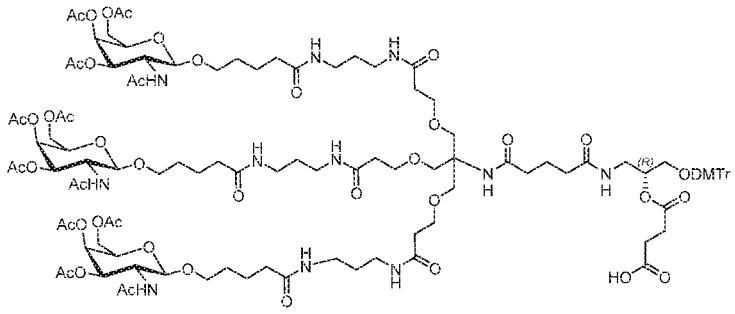
[0288] To a solution of **G-4** (8.90 g, 17.53 mmol, 1.05 equiv.) in N, N-dimethylformamide (300 mL) with an inert atmosphere of nitrogen, was added N, N-diisopropylethylamine (6.47 g, 50.16

mmol, 3.00 equiv.) at room temperature. To this was added O-Benzotriazole-N, N, N-etramethyl-uronium-hexafluorophosphate (7.10 g, 18.73 mmol, 1.12 equiv.) at room temperature. The resulting solution was stirred for 15 min at room temperature. To the mixture was added G-5 Ref (*Nucleic Acids Research, 2014, 42, (13) 8796–8807),* (30 g, 16.72 mmol, 1.00 equiv.) at room temperature. The resulting solution was allowed to react, with stirring, overnight at room temperature. The resulting mixture was concentrated under reduced pressure. The crude product was purified by Flash-Prep-HPLC with the following conditions (IntelFlash-1): Column, C18 silica gel; mobile phase, acetonitrile /water with 0.04% NH4HCO3 (20% acetonitrile up to 70% in 15 min); Detector, UV 210 nm. This resulted in 20.1 g (53%) of **G-6** as a white solid. MS m/z $[M+H]^+$ (ESI): 2283.

Synthesis of G-7



G-6

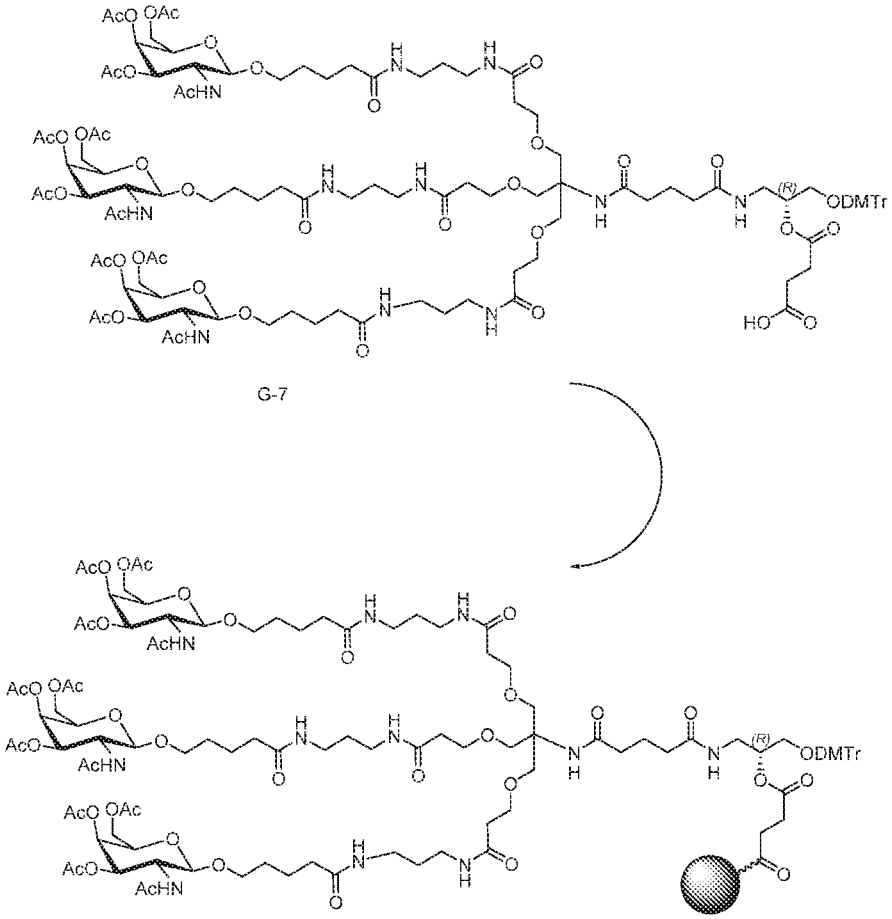


G-7

[0289] To a solution of **G-6** (25 g, 10.96 mmol, 1.00 equiv.) in dichloromethane (750 mL) with an inert atmosphere of nitrogen, was added triethylamine (4.98 g, 49.21 mmol, 4.49 equiv.) at room temperature. To this was added 4-dimethylaminopyridine (1.33 g, 10.89 mmol, 0.99 equiv.) at room temperature. To the mixture was added oxolane-2, 5-dione (3.29 g, 32.88 mmol, 3.00 equiv.) at room temperature. The resulting solution was stirred overnight at room temperature. The resulting mixture was concentrated under reduced pressure. The crude product

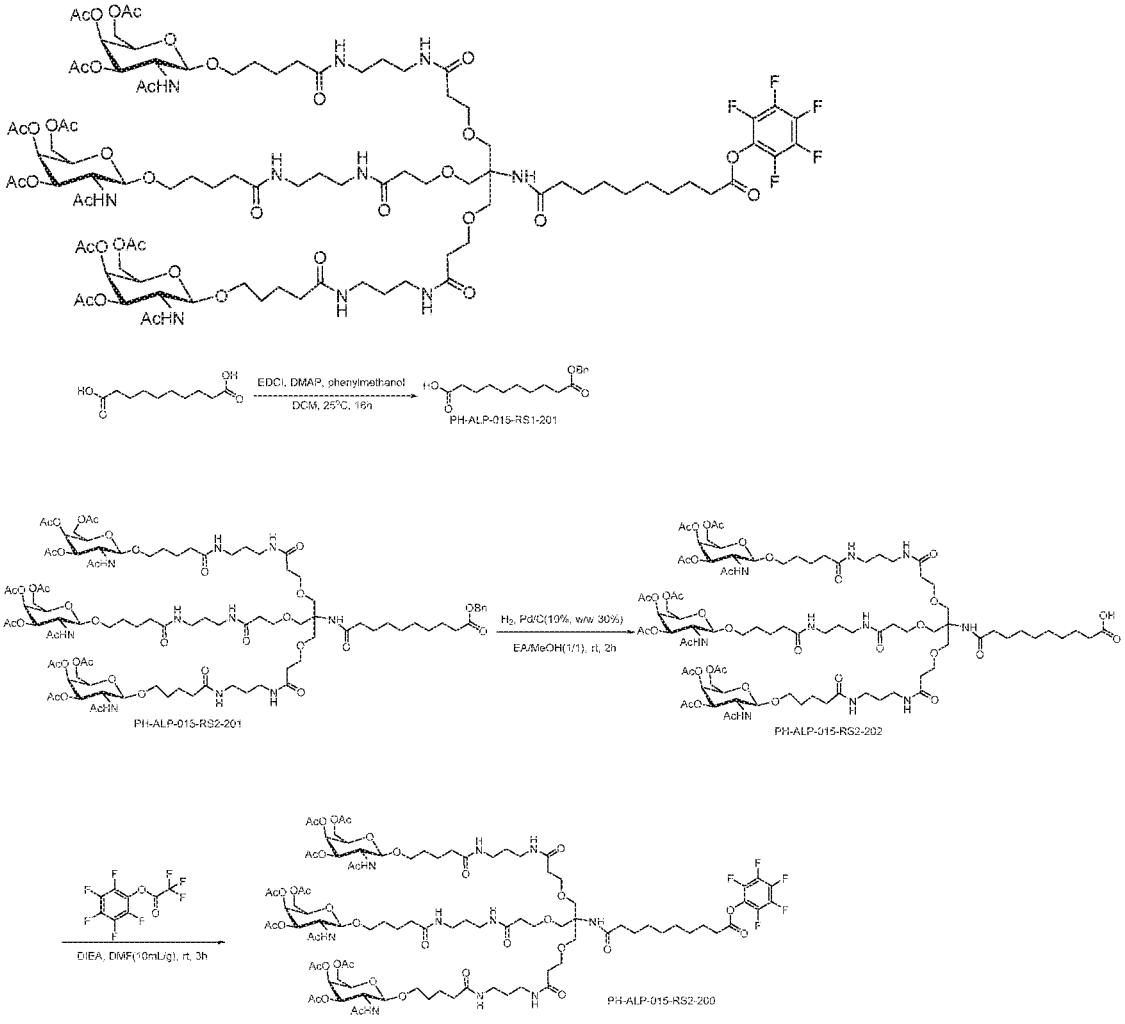
was purified by Flash-Prep-HPLC with the following conditions: Column, C18 silica gel; mobile phase, acetonitrile/water with 0.04% NH_4HCO_3 (20% acetonitrile up to 50% in 20 min); Detector, UV 230 nm. This resulted in 15.83 g (61%) of **G-7** as a white solid as ammonium salt. MS m/z [M/2+NH4]+ (ESI): 1210.

Synthesis of GalNAc-2-solid support-GPG

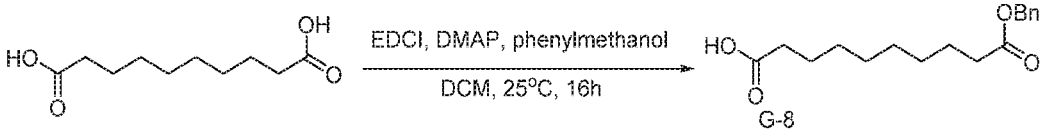


[0290] The G-7 was loaded onto the CPG by following the procedures described in Biotechniques.,1988 Sep;6(8):768-75 using HBTU/TEA to give GalNAc-2- CPG (53 µmol/g).

Synthesis of GalNAc-6



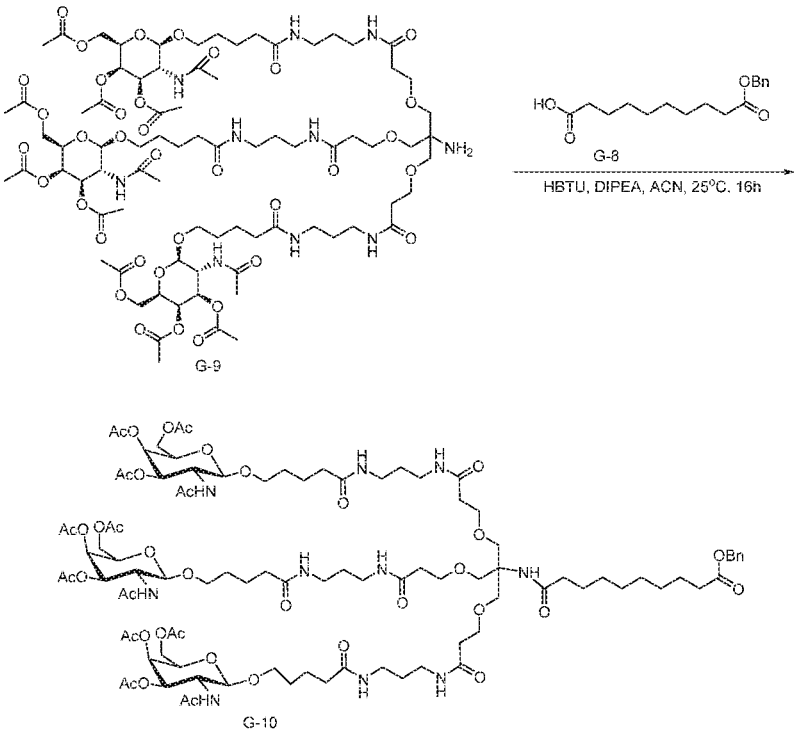
Synthesis of G-8



[0291] To a solution of decanedioic acid (100 g, 494.4 mmol, 1.00 equiv.) in dichloromethane (2000 mL), was added 4-dimethylaminopyridine (18.1 g, 148.2 mmol, 0.30 equiv.) at room temperature. To this was added N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (114 g, 594.7 mmol, 1.20 equiv.) at room temperature. The resulting solution was stirred for 1 h at room temperature. To the mixture was added Benzyl alcohol (64.1 g) dropwise with stirring at 0°C. The resulting solution was allowed to react, with stirring, overnight at room temperature. The resulting mixture was washed with saturated aqueous sodium chloride. The mixture was

dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product (100 g) was purified by Flash-Prep-HPLC with the following conditions (IntelFlash-1): Column, C18 silica gel; mobile phase, water and acetonitrile (60% acetonitrile up to 100% in 12 min and hold 100% for 5 min); Detector, UV 210 nm. This resulted in 60.7 g (42%) of **G-8** as a white solid. MS m/z [M+H]+ (ESI): 293.

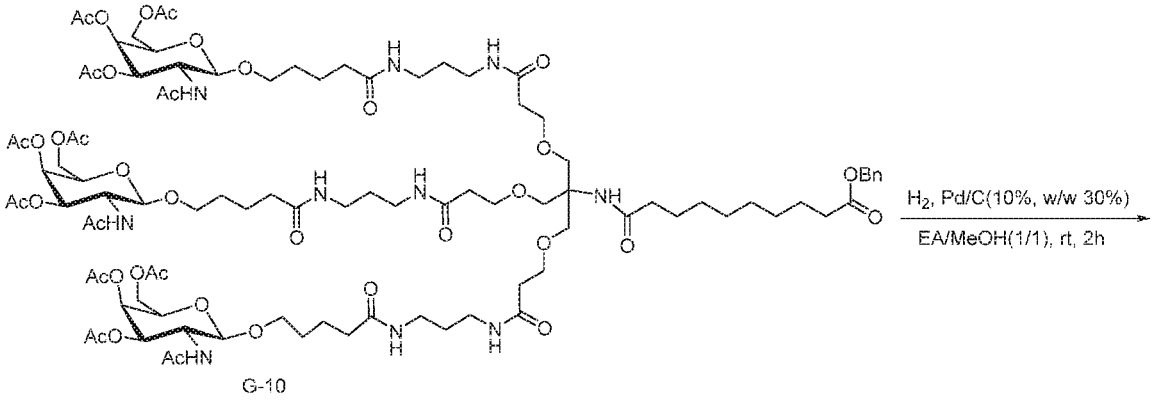
Synthesis of G-10



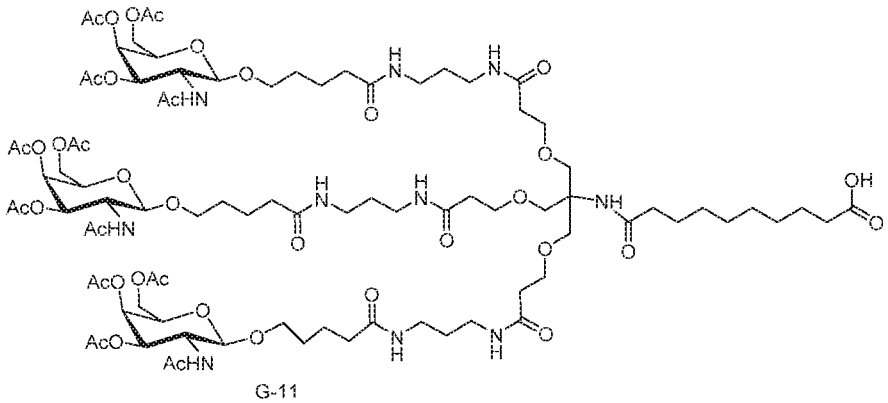
[0292] To a solution of **G-8** (4.48 g, 15.32 mmol, 1.50 equiv.) in acetonitrile (320 mL) was added O-Benzotriazole-N,N,N-etramethyl-uronium-hexafluorophosphate (5.84 g, 15.40 mmol, 1.50 equiv.), N,N-Diisopropylethylamine (3.96 g, 30.64 mmol, 3.00 equiv.). The resulting solution was stirred for 1 h at 25°C. This was followed by the addition of **G-9** (18.4 g, 10.26 mmol, 1.00 equiv.). The resulting solution was stirred for 16 h at 25 °C, and then concentrated under vacuum. The crude product was purified by Flash with the following conditions: Column, C18 silica gel; mobile phase, acetonitrile in water = 10% increasing to 70% within 15 min; Detector, UV 210 nm. This resulted in 12 g (57%) of **G-10** as a white solid. H-NMR (DMSO, 400MHz, *ppm*): 7.74-7.83 (m, 9H), 7.31-7.37 (m, 5H), 6.97 (s, 1H), 5.21 (d, J = 3.3 Hz, 3H), 5.07 (s, 2H), 4.98 (dd, J = 11.2 Hz, 3.4 Hz, 3H), 4.49 (d, J = 8.4 Hz, 3H), 4.04 (s, 9H), 3.83-3.99

(m, 3H), 3.67-3.72 (m, 3H), 3.52-3.55 (m, 12H), 3.37-3.43 (m, 3H), 2.99-3.05 (m, 12H), 2.25-2.35 (m, 8H), 2.12 (s, 9H), 1.99-2.11 (m, 17H), 1.92 (s, 9H), 1.77 (s, 9H), 1.40-1.53 (m, 22H), 1.19-1.25 (m, 8H).

Synthesis of G-11



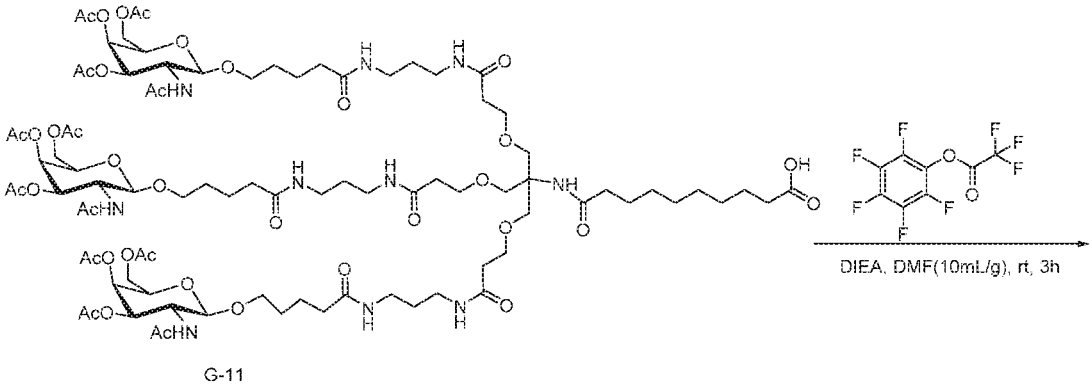
G-10



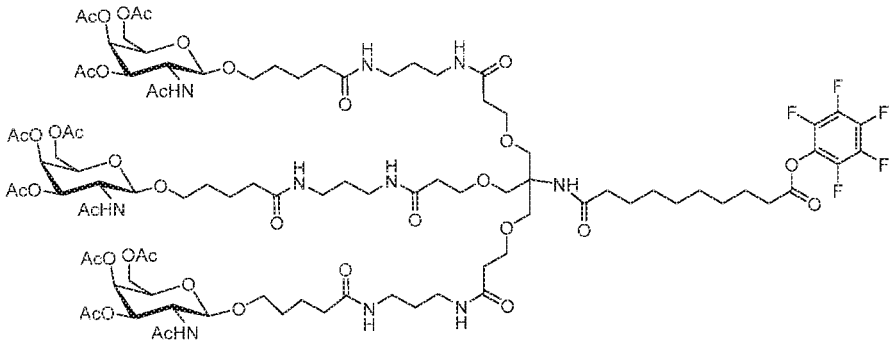
G-11

[0293] To a solution of **G-10** (5 g, 2.45 mmol, 1.00 equiv.) in methanol/ ethyl acetate (100 mL, v/v=1:1) was added 10% palladium carbon (1.5 g, 10%). The flask was evacuated and flushed five times with hydrogen. The mixture was stirred 2 h at room temperature under an atmosphere of hydrogen. The solids were filtered out. The resulting mixture was concentrated under vacuum. This resulted in 4 g (82%) of **G-11** as a white solid.

Synthesis of GalNAc-6



G-11

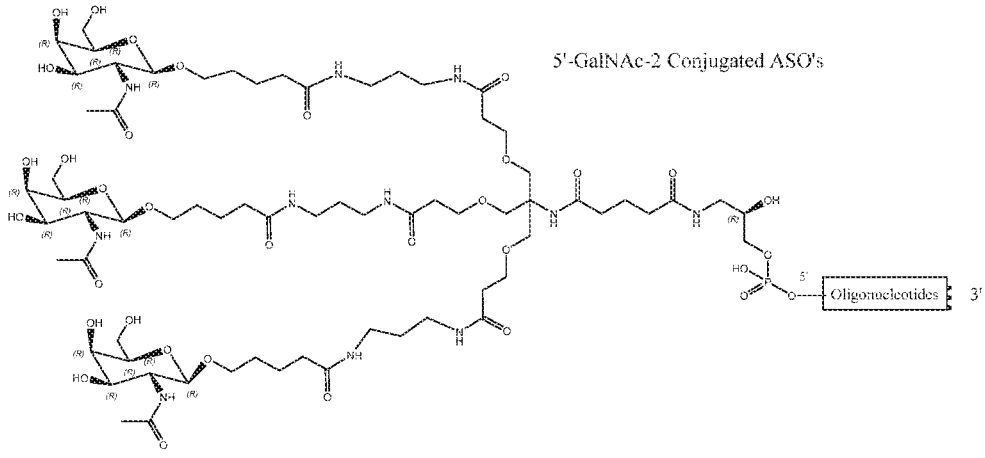


GalNAc-6

[0294] To a solution of **G-11** (6.3 g, 3.18 mmol, 1.00 equiv.) in N,N-dimethylformamide (63 mL) was added N,N-diisopropylethylamine (1.0 g, 7.95 mmol, 2.50 equiv.). This was followed by the addition of pentafluorophenyl 2,2,2-trifluoroacetate (1.33 g, 4.77 mmol, 1.50 equiv.) dropwise with stirring at 0°C. The resulting solution was stirred for 3 h at 25°C. The resulting mixture was concentrated under vacuum. The crude product was purified by Flash with the following conditions: C18 gel column, eluent A water, eluent B acetonitrile; gradient: 20% up to 80% within 15 min, 100% maintained 3 min; Detector, UV 210 nm. This resulted in 5 g (73%) of **GalNAc-6** as a white solid. MS m/z $[M/2+H]^+$ (ESI): 1073; H-NMR (DMSO, 300MHz, *ppm*): 7.71-7.80 (m, 9H), 6.98 (s, 1H), 5.22 (d, J = 3.3 Hz, 3H), 4.99 (dd, J = 11.1 Hz, 3.3 Hz, 3H), 4.50 (d, J = 8.4 Hz, 3H), 4.02 (s, 9H), 3.82-3.92 (m, 3H), 3.69-3.74 (m, 3H), 3.52-3.56 (m, 12H), 3.39-3.44 (m, 3H), 3.03 (s, 12H), 2.75-2.79 (m, 2H), 2.28 (t, J = 6.3 Hz, 6H), 2.00-2.10 (m, 26H), 1.89 (s, 9H), 1.77 (s, 9H), 1.64-1.68 (m, 2H), 1.25-1.53 (m, 28H); F-NMR (DMSO, 162MHz, *ppm*): -153.60, -153.67, -153.68, -153.69, -158.05, -158.14, -158.22, -162.53, -162.60, -162.62, -162.69, -162.70.

GalNAc conjugation

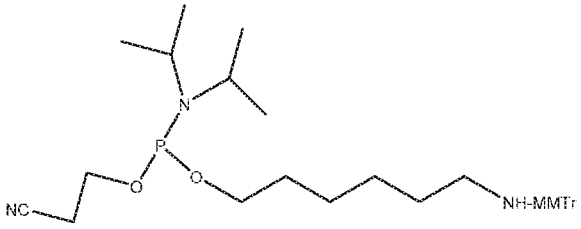
[0295] For making the 5' GalNAc Conjugated oligomer with the following modifications: 2'-F-NPS-PS-2'-F-NPS ; 2'-F-NP-PS-2'-F-NP; 2'-OMe-NP-PS-2'-OMe-NP; 2'-OMe-NPS-DNA-PS-2'-OMe-NPS, 2'-OEt-NPS-DNA-PS-2'-OEt-NPS and 2'-MOE-NPS-DNA-PS-2'-MOE-NPS the synthesis was carried out on a 10 to 200 µM scale in a 5' to 3' direction with the 5'-phosphoramidite monomers diluted to a concentration of 0.1 M in anhydrous CH_3CN in the presence of 5-(benzylthio)-1H-tetrazole activator (coupling time 2.0-4.0 min) to a GalNAc 2-CPG. The coupling cycle with modified protocols followed by standard capping, oxidation, and deprotection afforded modified oligonucleotides. The stepwise coupling efficiency was more than 98%. The DDTT (dimethylamino-methylidene) amino)-3H-1, 2, 4-dithiazaoline-3-thione was used as the sulfur-transfer agent for the synthesis of oligoribonucleotide phosphorothioates. The 0.2 M Phenyls acetyl disulfide (PADS) in Lutidine:Acetonitrile (1:1) was used as sulfurizing agent in large-scale synthesis (Akta OP-100). Oligonucleotide-bearing solid supports were heated at room temperature with aqueous ammonia/Methylamine (1:1) solution for 3 h in shaker to cleavage from support and deprotect the base labile protecting groups.



3'-C6NH2-NPS-PS-NPS-(Precursor) synthesis

[0296] For making the 3' GalNAc Conjugated oligomers with the following modifications: 2'-F-NPS-PS-2'-F-NPS ; 2'-F-NP-PS-2'-F-NP; 2'-OMe-NP-PS-2'-OMe-NP; 2'-OMe-NPS-DNA-PS-2'-OMe-NPS, 2'-OEt-NPS-DNA-PS-2'-OEt-NPS and 2'-MOE-NPS-DNA-PS-2'-MOE-NPS ASOs were synthesized at 10 µmol scale using universal support (Loading 65 µmol/g). The synthesis procedure is same as described above. At the 3'-terminal to introduce C6-NH2 linker

the 6-(4-Monomethoxytritylamino)hexyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite in 0.1 M Acetonitrile was used with coupling time 10 min. The Oligonucleotide-bearing solid supports were heated at room temperature with aqueous ammonia/Methylamine (1:1) solution for 3 h in shaker to cleavage from support and deprotect the base labile protecting groups. After IEX purification and desalting the C6-NH2 modified ASO's can be used to perform post synthesis conjugation.

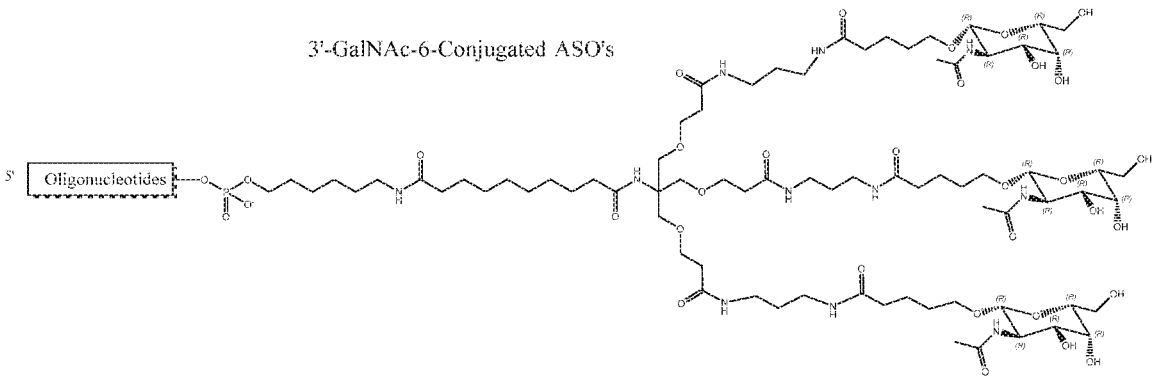


5'-Amino-Modifier C6

6-(4-Monomethoxytritylamino)hexyl-(2-cyanoethyl)-(N,N-diisopropyl)- phosphoramidite

3'-GalNAc NPS-PS-NPS-ASO synthesis (Post Synthesis Conjugation)

[0297] The 3'-C6-NH_2 modified ASOs were dissolved in 0.2 M Sodium bicarbonate buffer, pH 8.5 (0.015 mM) and 5-7 mol equivalent of GalNAc-6 ester dissolved in DMSO was added. The reaction mixture was stirred at room temperature for 4 h. The sample was analyzed to confirm if any unreacted amino modified ASO's is present. To this aqueous ammonia (28 wt. %) was added (5× reaction volume) and stirred at room temperature for 2-3 h. Reaction mixture concentrated under reduced pressure and residue dissolved in water and purified by HPLC on a strong anion exchange column.



3'-GalNAc6 Conjugation

Conc. Of Oligo's	Equivalent of GalNAc 6 PFP ester	Temp (°C)	% Conversion to 3' GalNAc ASO
0.015 mM	5	25	75
0.0076 mM	7	25	80
0.0076 mM	4	25	65

Quantitation of Crude Oligomer or Raw Analysis

[0298] Samples were dissolved in deionized water (1.0mL) and quantitated as follows: Blanking was first performed with water alone (1.0 mL) 20ul of sample and 980 µL of water were mixed well in a microfuge tube, transferred to cuvette and absorbance reading obtained at 260 nm. The crude material is dried down and stored at -20 °C.

Crude HPLC/LC-MS analysis

[0299] The 0.1 OD of the crude samples were submitted for crude MS analysis. After Confirming the crude LC-MS data then purification step was performed.

HPLC Purification

[0300] The Phosphoramidate (NP) and Thiophosphoramidate (NPS) modified oligonucleotides with and without GalNAc conjugates were purified by anion-exchange HPLC. The buffers were 20 mM sodium phosphate in 10 % CH_3CN, pH 8.5 (buffer A) and 20 mM sodium phosphate in 10% CH_3CN, 1.8 M NaBr, pH 8.5 (buffer B). Fractions containing full-length oligonucleotides were pooled, desalted, and lyophilized.

Desalting of Purified Oligomer

[0301] The purified dry oligomer was then desalted using Sephadex G-25 M (Amersham Biosciences). The cartridge was conditioned with 10 mL of deionized water thrice. Finally the purified oligomer dissolved thoroughly in 2.5mL RNAse free water was applied to the cartridge with very slow drop wise elution. The salt free oligomer was eluted with 3.5 ml deionized water directly into a screw cap vial.

IEX HPLC and Electrospray LC/MS Analysis

[0302] Approximately 0.10 OD of oligomer is dissolved in water and then pipetted in special vials for IEX-HPLC and LC/MS analysis. Analytical HPLC and ES LC-MS established the integrity of the oligonucleotides. The purity and molecular weight were determined by HPLC analysis (60 °C, IEX- Thermo DNAPac PA-100, A- 25 mM sodium phosphate 10% acetonitrile pH 11, B- 1.8 M NaBr 25 mM sodium phosphate 10% acetonitrile pH 11; RPIP- Waters XBridge OST C18, A- 100 mM HFIP 7 mM TEA B- 7:3 methanol/acetonitrile) and ESI-MS analysis using Promass Deconvolution for Xcalibur (Novatia, Newtown, PA). All oligonucleotides in the following tables were synthesized, and reference to molecular weights in the tables are actual measured weights that may have an error of MW, amu +/-2.

Stability Testing of Complexed Oligonucleotides

In embodiments, the disclosed oligonucleotides display an increased affinity for a target nucleic acid sequence compared to an unmodified oligonucleotide of the same sequence. For example, in some sequences the disclosed oligonucleotides has a nucleobase sequence that is complementary or hybridizes to a target nucleic acid sequence at a higher affinity than an unmodified oligonucleotide of the same sequence. In embodiments, the disclosed oligonucleotide complexed with a complementary target nucleic acid sequence has a melting temperature T_m of >37 °C. The complex may be formed under physiological conditions or nearly physiological conditions such as in phosphate-buffered saline (PBS). In embodiments, the Tm of the complex is >50 °C. In embodiments, the Tm of the complex is 50-100 °C. In embodiments, the Tm of a disclosed oligonucleotide duplexed with a target nucleic acid sequence under physiological conditions or nearly physiological conditions is >50 °C.

[0303] In certain embodiments, the target nucleic acid sequence may be selected from a nucleic acid sequence of a known viral DNA or RNA sequence such as the HBV genome.

[0304] In embodiments, the disclosed oligonucleotides display an affinity for at least one of the following six sequences of the HBV genome or its RNA equivalents and/or display stability complexed to at least one of the following six sequences of the HBV genome (Table E) or its RNA equivalents (Table F). In embodiments, the oligonucleotide complexed with a complementary HBV genome sequence has a melting temperature (Tm) of >37 °C. The HBV genome may be an RNA sequence such as DR-1 and/or DR-2 RNA sequence. The complex may

be formed under physiological conditions or nearly physiological conditions such as in phosphate-buffered saline (PBS). In embodiments, the Tm of the complex is >50 °C. In embodiments, the Tm of the complex is 50-100 °C. In embodiments, the Tm of a disclosed oligonucleotide duplexed with an HBV RNA under physiological conditions or nearly physiological conditions is >50 °C.

In Vitro Testing of Oligonucleotides

[0305] Two HBV cell lines were used to assess the in vitro potency of oligonucleotides: HepG2.2.15 (2215) and HepG2.117 (2117). HBsAg reduction in tissue culture supernatant (sup) as well as cytotoxicity was measured using HepG2.2.15 cell. HBV DNA reduction in the sup as well as intracellular fraction was measured in HepG2.117 cell.

[0306] HepG2.2.15 cell line is a stable cell line with four integrated HBV genomes. The cells were grown at 37°C in an atmosphere of 5% CO_2 in Dulbecco's modified Eagle's medium supplemented with 10% FCS, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 2% glutamine. The day before the dosing, $2.5x10^4$ cells/well were plated in collagen coated 96 well plates and incubated overnight. On the day of dosing, serially diluted oligomers were transfected into the cells with Lipofectamine RNAiMax (Thermo Fisher, Waltham, MA) following manufacturer's protocol. Duplicates were made for each drug concentration and each oligo was set up for both EC50 measurement and CC50 measurement. Three days after transfection, the supernatant (sup) was collected and used in HBsAg ELISA (AutoBio, China) for EC50 calculation. For CC50 measurement, CellTiter-Glo® (Promega, Madison, WI) was used in the assay following manufacturer's instruction.

[0307] HepG2.117 is a stable hepatoma cell line harboring an integrated 1.05 copy of the HBV genome (subtype ayw) under regulation of TetOFF (induction of transcription in the absence of tetracycline or its homolog doxycycline). The cells were grown at 37°C in an atmosphere of 5% CO_2 in DMEM/F12 media supplemented with 10% FCS, 100 IU/ml penicillin, 100 µg/ml streptomycin, 2% glutamine, 250 µg/ml G418, and 2 µg/ml Tetracycline. The day before the dosing, the cell media-containing Tetracycline was removed, the cells washed to remove the residual Tetracycline and plated at $2.5x10^4$ cells/well with treatment media (DMEM/F12 containing 2% Tet-system approved FBS 100 IU/ml penicillin, 100 µg/ml streptomycin, and 2% glutamine) in collagen coated 96 well plates. The cells were then incubated overnight. On the

day of experiment, serially diluted oligomers were transfected into the cells with Lipofectamine RNAiMax (Thermo Fisher, Waltham, MA) following manufacturer's protocol. Duplicates were made for each drug concentration and each oligo was set up for both EC50 measurement and CC50 measurement. Four days after the transfection, the sup was collected to be used in HBV DNA qPCR directly. The HBV DNA from the cells was isolated with MagMAX™ Total Nucleic Acid Isolation Kit (Thermo Fisher) and then applied in qPCR as template. HBV subtype ayw DNA (accession number V01460) sequence was used to design (Primer Express, Thermo Fisher) the forward primer (5′-TTG CCT TCT GAC TTC TTT CCT TCT-3′), reverse primer (5′-TGC CTG AGT GCT GTA TGG TGA G-3′) and the fluorogenic TaqMan® probe (5′-TCG GGA AGC CTT AGA GTC TCC TGA-3′) labelled with FAM (6-carboxyfluoresceine) in 5′ and with TAMRA (6-carboxytetramethylrhodamine) in 3′. These primers and probe were used to carry out quantitative real-time PCR with AmpliTaq Gold DNA polymerase (Perkin-Elmer Life Science, Waltham, MA). The conditions for this reaction were as follows: 1 cycle, hot-start at 95°C for 10 min followed by 50 cycles of denaturation (95°C for 15 s) and annealing/ polymerization (59°C for 1 min).

Infectious HBV system in primary human hepatocyte

[0308] Cryopreserved primary human hepatocytes (PHH)were thawed and plated in 24 well plates at 200,000 cells/well. The cells were allowed to recover overnight at 37°C 5% CO_2. The cells were infected O/N (37°C/5% CO_2) with HBV at moi 50-100. After infection for overnight, the viral inoculum is removed and the cells are washed three times with prewarmed wash medium. Then refill with fresh PHH culturing medium. The medium is replaced with 450µl fresh medium. Add 50ul transfect mixture. Dilute oligomers in Opti-MEM I (Life Technology, Cat#: 31985-070) to 20x of final concentration, mix with equal volume Opti-MEM I containing Lipofectamine RNAiMAX (Invitrogen, Cat#: 13778-150), pipet 3 times and incubate for 10-20min at room temperature. Add 50ul oligo:RNAiMAX mixture into the wells, tap the plates a few times with hands. Put the plates back to incubator. On the day of assay, Harvest supernatant for HBsAg and HBeAg ELISA, cell for cell viability. HBsAg ELISA was described in above section. For HBeAg, method from Autobio Diagnostics (CL0312-2) was used.

In Vivo Testing of Oligonucleotides

[0309] AAV/HBV is a recombinant AAV carrying replicable HBV genome. Taking advantage of the highly hepatotropic feature of genotype 8 AAV, the HBV genome can be efficiently delivered to the mouse liver cells. Infection of immune competent mouse with AAV/HBV can result in long-term HBV viremia, which mimics chronic HBV infection in patients. The AAV/HBV model can be used to evaluate the in vivo activity of various types of anti-HBV agents. Mice were infected with AAV-HBV on day -28 of the study. The test articles or negative control (PBS) were dosed subcutaneously (unless specified otherwise) three times on days 0, 2 and 4 at the specified dose levels. Or they can be injected as single dose at specified dose levels on day 0. The positive control, entecavir (ETV), for HBV DNA, but not for HBV antigens, was dosed orally every day. Serum HBV S antigen (HBsAg) and E antigen (HBeAg) were assayed through ELISA and HBV DNA through real time PCR. ELISA methods and qPCR method have been described in the in vitro assay sections above.

[0310] The following statements describe how the data in Table 1-43 were generated. For all of the in vitro HBsAg Cell line EC50 and CC50 data, the method for HepG2.2.15 was used and accordingly, "2215" was labeled in the columns or rows where the data was shown. For all of the in vitro HBV DNA Cell line EC50 and CC50 data, the method for HepG2.117 was used and accordingly, "2117" was labeled in the columns or rows where the data was shown. For all in vitro HBsAg as well as HBeAg EC50 data tested in HBV/PHH infectious system, PHH method was used and accordingly "PHH" was labeled in the columns or rows where the data was shown. For in vivo AAV-HBV mouse model results, method in in vivo section above was applied. The Maximum HBsAg (or HBeAg) reduction was described as nadir (unit Log reduction) and the nadir was labeled in the columns or rows where the data was shown. Two ASOs were often compared for their nadir. If value other than nadir was compared, they will be indicated in the text.

Method of Treatment

[0311] An adult human suffering from HBV infection is administered intravenously a therapeutically effective compound of the present disclosure, for example, a compound selected from Table 1-43. Treatment is continued until one or more symptom of HBV is ameliorated, or for example, serum HBV S antigen (HBsAg) and/or E antigen (HBeAg) levels are reduced.

[0312] An adult human suffering from HBV infection is administered subcutaneously a therapeutically effective compound of the present disclosure, for example, a compound selected from Table 1-43. Treatment is continued until one or more symptom of HBV is ameliorated, or for example, serum HBV S antigen (HBsAg) and/or E antigen (HBeAg) levels are reduced.

[0313] In the following tables, A through J corresponds to the following:

A) 0.05-10 nM;
B) 10-100 nM;
C) above 100 nM;
D) 0.1-5.0 nM;
E) 5.1-10.0 nM;
F) 10.1-21 nM;
G) 20-100
H) 10-1000
I)>1,000
J)>10,000.

Table 1: Chimeric oligonucleotide with PS and 2'-O-Me Modifications

#ID	Sequence (5'-3')	2215 HBsAg EC50 (nM)	2215 CC50 (nM)	Max HBsAg Log reduction* (nadir)	Molecular Weight (MW)
101	5' mGpsmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmApsmApsmGpsmUpsmGpsmC-3'	A	J		6967.66
102	5' mGpsmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmApsmApsmGpsmUpsmGpsmCps-Chol-3'	B	J		7739.69
103	5' mGpsmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmApsmApsmGpsmUpsmGpsmC-GalNAc-3'	B	J	2	8728.57

* Log Reduction post 3x30mg/kg SC

[0314] FIGs. 1A-C show results of 2-week testing of a compound of the present disclosure *in vivo* in an AAV/HBV mouse model. AAV/HBV is a recombinant Adeno-associated virus (AAV) carrying replicable HBV genome. Taking advantage of the highly hepatotropic feature of genotype 8 AAV, the HBV genome can be efficiently delivered to the mouse liver cells. Infection of immune competent mouse with AAV/HBV can result in long-term HBV viremia,

which mimics chronic HBV infection in patients. The AAV/HBV model can be used to evaluate the in vivo activity of various types of anti-HBV agents. Mice were infected with AAV-HBV on day-28 of the study. The test articles or negative control (PBS) were dosed subcutaneously (unless specified otherwise) three times on days 0, 2 and 4 at the specified dose levels. The positive control entecavir (ETV, for HBV DNA, but not for HBV antigens) was dosed orally every day. Serial blood collections were carried out on the days shown in the figures. Serum HBV S antigen (HBsAg) and E antigen (HBeAg) were assayed through ELISA and HBV DNA through real time PCR. In FIG. 1, three test articles #101, #102 (3' Cholesterol conjugated form of #101) and #103 (3' GalNAc conjugated form of #101) were tested along with ETV.

[0315] FIG. 1A shows HBsAg serum levels. ETV is known to reduce HBV DNA but has no effects on either HBsAg or HBeAg. GalNAc conjugated #101 reduced HBsAg ~ 2 log while unconjugated #101 and Cholesterol conjugated #102 had very little effect.

[0316] FIG. 1B shows HBeAg serum levels; and FIG. 1C shows DNA serum levels. The patterns for these three oligomers on HBeAg were very similar to that of HBsAg. The max HBeAg drop for #103 was ~ 0.7 log.

[0317] FIG. 1C shows DNA serum levels. All three oligomers reduced HBV DNA in mouse serum with GalNAc conjugated #103 being the most potent compound (max HBV DNA reduction on day 14 was ~ 3 log comparing with day 0 baseline). The positive control ETV also showed max 3 log drop in HBV DNA.

[0318] FIGs. 2A-B show HBsAg serum levels for a GalNAc conjugated compound of the present disclosure as a SC and an IV administration in an *in vivo* mouse model. FIG. 2A show results for IV administration; FIG. 2B shows results for SC administration. The SC delivery showed slightly higher degree of HBsAg than the IV delivery with the same dosage

Table 2

#ID	Sequence (5'-3')	2215 EC50 (nM)	2215 CC50 (nM)	Max HBsAg Log reduction (nadir)*	MW

104	5' mGpsmApsmUpsmUpsmApsmGpsGpsCpsApsGps ApsGpsGpsTpsmGpsmApsmApsmApsmApsmAps mG 3'	B	J		7275.92
105	5' mGpsmApsmUpsmUpsmApsmGpsGpsCpsAps GpsApsGpsGpsTpsmGpsmApsmApsmApsmApsm ApsmG-Chol-3	A	J		8031.88
106	5' mGpsmApsmUpsmUpsmApsmGpsGpsCpsAps GpsApsGpsGpsTpsmGpsmApsmApsmApsmApsm ApsmG-GalNAc-3	C	J	0.8	9036.82

* Log Reduction post 3x30mg/kg SC

[0319] FIG. 3 shows HBsAg reduction levels for GalNAc conjugated compounds of the present disclosure (#106, #109, #162 and #159) via subcutaneous delivery in an *in vivo* AAV-HBV mouse model. The max HBsAg reductions for these ASO were similarly ~ 1 Log.

Table 3

#ID	Sequence (5'-3')	2215 EC50 (nM)	2215 CC50 (nM)	Max HBsAg Log reduction (nadir)*	MW
107	5' mGpsmApsmUpsmUpsmApsGpsGpsCpsApsGps ApsGpsGpsTpsmGpsmApsmApsmApsmApsmApsm G 3'	B	J		7245.89
108	5' mGpsmApsmUpsmUpsmApsGpsGpsCpsApsGps ApsGpsGpsTpsmGpsmApsmApsmApsmApsmApsm G-Chol-3	A	J		8001.85
109	5' mGpsmApsmUpsmUpsmApsGpsGpsCpsApsGps ApsGpsGpsTpsmGpsmApsmApsmApsmApsmApsm G-GalNAc-3	C	J	1	9006.80

* Log Reduction post 3x30mg/kg SC

Table 4

#ID	2215 HBsAg EC50 (nM)	2117 sup HBVDNA (EC50 nM)	2117 Intra HBVDNA EC50(nM)	2215 CC50 (nM)	MW
110	B	F	F	J	7305.95
111	B	E	E	J	7320.96
112	B	D	D	J	7350.99
113	B	D	D	J	7350.99

114	A	D	D	J	7381.02
115	B	D	E	J	7275.92
116	B	F	E	J	7290.94
117	A	D	D	J	7320.97
118	B	E	D	J	7320.97
119	A	D	D	J	7351.00

#ID	Sequence (5'-3')
110	5' mGpsmDAPpsmUpsmUpsmDAPpsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmApsmApsmG 3'
111	5' mGpsmApsmUpsmUpsmApsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmDAPpsmDAPpsmmDAPpsmG 3'
112	5' mGpsmApsmUpsmUpsmApsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmDAPpsmDAPpsmDAPpsmDAPpsmDAPpsmG 3'
113	5' mGpsmDAPpsmUpsmUpsmDAPpsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmDAPpsmDAPpsmDAPpsmG 3'
114	5' mGpsmDAPpsmUpsmUpsmDAPpsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmDAPpsmDAPpsmDAPpsmDAPpsmDAPpsmG 3'
115	5' mGpsmDAPpsmUpsmUpsmDAPpsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmApsmApsmG 3'
116	5' mGpsmApsmUpsmUpsmApsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmDAPpsmDAPpsmDAPpsmG 3'
117	5' mGpsmApsmUpsmUpsmApsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmDAPpsmDAPpsmDAPpsmDAPpsmDAPpsmG 3'
118	5' mGpsmDAPpsmUpsmUpsmDAPpsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmDAPpsmDAPpsmDAPpsmG 3'
119	5' mGpsmDAPpsmUpsmUpsmDAPpsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmDAPpsmDAPpsmDAPpsmDAPpsmDAPpsmG 3'

Table 5

#ID	Sequence (5'-3')	2215 EC50 (nM)	2215 CC50 (nM)	MW
120	5' mGpsmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmApsmDAPpsmGpsmUpsmGpsmC-3	A	J	6982.68
121	5' mGpsmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmDAPpsmDAPpsmGpsmUpsmGpsmC-3	A	J	6997.69
122	5' mGpsmCpsmApsmGpsmDAPpsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmDAPpsmDAPpsmGpsmUpsmGpsmC-3	A	J	7012.71
123	5' mGpsmCpsmDAPpsmGpsmDAPpsmGpsGpsTpsGpsApsApsGpsmCpsmGpsmDAPpsmDAPpsmGpsmUpsmGpsmC-3	A	C	7027.72

Table 6

#ID	HBsAg EC50 (nM)	CC50 (nM)	Sequence (5'-3')	Max HBsAg Log reduction (nadir)*	MW
159	B	J	5'mApsmApsmGpsmApsmGpsApsGpsGpsTpsGps5meCpsGps5meCps5meCps5meCps5memCpsmGpsmUpsmGpsmG –GalNAc 3'	1	8630.55
160	B	J	5' mGpsmGpsmUpsmGpsmApsApsGps5meCpsGpsApsApsGpsTpsGps5mCpsmAps5memCpsmAps5memeCps mG –GalNAc 3'	1.9	8624.54
161	B	J	5'mUpsmGpsmGps5memCpsmAps5meCpsTpsApsGpsTpsApsApsAps5meCpsTpsmGpsmApsmGps5memCps5memC –GalNAc 3'	1.9	8548.52
162	B	J	5'5memCpsmUpsmApsmGmGpsApsGpsTpsTps5meCps5meCpsGps5meCpsApsGpsmUpsmApsmUpsmGpsmG–GalNAc 3'	1	8553.45
163	B	J	5' mApsmGpsmApsmGpsmGpsTpsGps5meCpsGps5meCps5meCps5meCps5meCpsGpsTpsmGpsmGpsmUps5memCpsmG –GalNAc 3'	0.8	8611.53
164	B	J	5' mUps5memCps5memCpsmGps5memCpsApsGpsTpsApsTpsGpsGpsApsTps5meCpsmGpsmGps5memCps mApsmG –GalNAc 3'	1.9	8610.55
165	B	J	5' mUpsmGps5memCpsmApsmGpsApsGpsGpsTpsGpsApsApsGps5meCpsGpsmApsmApsmGpsmUpsmG–GalNAc 3'	2.8	8637.50
166	B	J	5' mApsmGpsmUps5memCps5memCpsAps5meCps5meCAps5meCpsGpsApsGpsTps5meCpsmUpsmApsmGps mAps5memC-GalNAc 3'	0.3	8507.51

* Log Reduction post 3x30mg/kg SC

[0320] FIG. 3 shows HBsAg reduction levels for GalNAc conjugated compounds of the present disclosure (#106, #109, #162 and #159) via subcutaneous delivery in an *in vivo* AAV-HBV mouse model. The max HBsAg reductions for these ASO were similarly ~ 1 Log.

[0321] FIGs. 4A-C show *in vivo* HBsAg, HBeAg and Serum HBV DNA data in an AAV-HBV mouse model for compounds of the present disclosure. #103, #164 and #165 when delivered SC, showed significant reductions in HBsAg, HBeAg and Serum HBV DNA in AAV-HBV mouse model. #103 also demonstrated dose response when dosed at two different dose levels. FIG. 4A shows HBsAg serum levels. FIG. 4B shows HBeAg serum levels. FIG. 4C shows HBV DNA levels.

[0322] FIGs. 4A-C show *in vivo* HBsAg, HBeAg and Serum HBV DNA data in an AAV-HBV mouse model for compounds of the present disclosure. FIG. 4A shows HBsAg serum levels. FIG. 4B shows HBeAg serum levels. FIG. 4C shows HBV DNA levels.

[0323] FIGs. 5A-C show *in vivo* HBsAg, HBeAg and serum HBV DNA data in an AAV-HBV mouse model for compounds of the present disclosure. #160, #161, #163, #166, #213 and #176 significantly reduced HBsAg, HBeAg and serum HBV DNA in AAV-HBV Mouse model. FIG. 5A shows HBsAg serum levels. FIG. 5B shows HBeAg serum levels. FIG. 5C shows HBV DNA levels.

Table 7

#ID	HBsAg 2215 EC50 (nM)	2215 CC50 (nM)	Base Sequence (5'-3')	Modified Sequence (5'-3')	MW
167	A	J	GCAGAGGTGAAGCGAAGTGC	5' fGnpsfCnpsfAnpsfGnpsfAnpsfGnpsGpsTpsGpsApsApsGpsfCnpsfGnpsfAnpsfAnpsfGnpsfUnpsfGnps-3-NH_2-fC	6785.38
168	A	J	GCAGAGGTGAAGCGAAGTGC	5' fGnpsfCnpsfAnpsfGnpsfAnpsfGnpsGpsTpsGpsApsApsGpsCpsfGnpsfAnpsfAnpsfGnpsfUnpsfGnps-3-NH_2-fC	6768.37
169	A	J	GCAGAGGTGAAGCGAAGTGC	5' fGnpsfCnpsfAnpsfGnpsfAnpsfGnpsGpsTpsGpsApsApsGpsCpsGpsfAnpsfAnpsfGnpsfUnpsfGnps-3-NH_2-fC	6751.37

Table 8

#ID	Sequence (5'-3')	HBsAg 2215 EC50 (μM)	HBsAg 2215 CC50 (μM)	MW
170	5' GnpCnpAnpGnpAnpGnpGpsTpsGpsApsApsGpsCnpGnpAnpAnpGnpTnpGnp-3 NH_2-C	A	H-I	6339.66
171	5' GnpsfCnpsfAnpsGnpsfAnpsGnpsGpsTpsGpsApsApsGpsfCnpsGnpsfAnpsfAnpsGnpsfTnpsGnps-3 NH_2-fC	A	H-I	6692.45
172	5' GnpfCnpfAnpGnpfAnpGnpGpsTpsGpsApsApsGpsfCnpGnpfAnpfAnpGnpfTnpGnp-3 NH_2-fC	A	H-I	6483.58

Table 9

#ID	Sequence (5'-3')	HBsAg 2215 EC50 (nM)	HBsAg 2215 CC50 (nM)	MW

173	5' GnpsafCnpsafAnpsGnspafAnpsGnpsGpsTpsGpsApsApsGpsafCnpsGnpsafAnpsafAnpsGnpsafUnpsGnpsafC	A	J	6677.43
174	5' GnpafCnpafAnpGnpafAnpGnpGpsTpsGpsApsApsGpsafCnpGnpafAnpafAnpGnpafUnpGnpafC	A	J	6468.57
175	5' GnpafCnpafAnpGnpafAnpGnpGpsTpsGpsApsApsGpsCpsGnpafAnpafAnpGnpafUnpGnpafC	A	J	6466.65

Table 10; Gapmer (2'Ome, 5MeC) with 5' GalNAc

#ID	Sequence (5'-3')	2215 HBsAg EC50 (nM)	Max HBsAg Log Reduction (nadir)*
204	5'-GalNAc-NHC6-psmUpsm5meCpsm5meCpsmGpsm5meCpsApsGpsTpsApsTpsGpsGpsApsTps5meCpsmGpsmGpsm5meCpsmApsmG 3'	C	1
205	5'-GalNAc-NHC6-psm5meCpsmUpsmApsmGpsmGpsApsGpsTpsTps5meCps5meCpsGps5meCpsApsGpsmUpsmApsmUpsmGpsmG 3'	B	1
206	5'-GalNAc-NHC6-psmApsmApsmGpsmApsmGpsApsGpsGpsTpsGps5meCpsGps5meCps5meCps5meCpsm5meCpsmGpsmUpsmGpsmG 3'	B	1
207	5' GalNAc-NHC6-psmApsmGpsmApsmGpsmGpsTpsGps5meCpsGps5meCps5meCps5meCps5meCpsGpsTpsmGpsmGpsmUpsm5meCpsmG 3'	B	0.5
208	5' GalNAc-NHC6-psmUpsmGpsm5meCpsmApsmGpsApsGpsGpsTpsGpsApsApsGps5meCpsGpsmApsmApsmGpsmUpsmG 3'		1.4

* Log Reduction post 3x30mg/kg SC

[0324] FIGs. 6A-C show *in vivo* HBsAg, HBeAg and serum HBV DNA data in an AAV-HBV mouse model for compounds of the present disclosure. #204, #205, #206, #207, #208 and #212 significantly reduced HBsAg, HBeAg and serum HBV DNA in AAV-HBV Mouse model. FIG. 56 shows HBsAg serum levels. FIG. 6B shows HBeAg serum levels. FIG. 6C shows HBV DNA levels.

Table 11: Pre-Poly-A

#ID	Sequence (5'-3')	2215 HBsAg EC50 (nM)	2215 CC50 (nM)	Max HBsAg Log Reduction (nadir)*	MW
209	mGpsmCpsmUpsmCpsmCpsmApsmApsApsTpsTpsCpsTpsTpsTpsmApsmUpsmApsmApsmGpsmG	A	J		6758.52
210	mGpsmCpsmUpsmCpsmCpsmApsApsApsTpsTpsCpsTpsTpsTpsmApsmUpsmApsmApsmGpsmG	A	J		6728.49
211	mGpsmCpsmUpsmCpsmCpsmApsApsApsTpsTpsCpsTpsTpsTpsApsmUpsmApsmApsmGpsmGpsmG	A	J		7073.77
212	mGpsmCpsmUpsmCpsmCpsmApsmApsApsTpsTpsCpsTpsTpsTpsmApsmUpsmApsmApsmGpsmG/GalNAc/			0.8	8519.43
213	mGpsmCpsmUpsmCpsmCpsmApsApsApsTpsTpsCpsTpsTpsTpsmApsmUpsmApsmApsmGpsmG/GalNAc/			1	8489.40
176	mGpsmCpsmUpsmCpsmCpsmApsApsApsTpsTpsCpsTpsTpsTpsApsmUpsmApsmApsmGpsmGpsmG/GalNAc/	B	I	1.2	8834.67
214	mGpsmCpsmUpsmCpsmCpsmApsmApsApsTpsTpsCpsTpsTpsTpsmApsmUpsmApsmApsmGpsmG/3CholTEG/				7514.48
215	mGpsmCpsmUpsmCpsmCpsmApsApsApsTpsTpsCpsTpsTpsTpsmApsmUpsmApsmApsmGpsmG/3CholTEG/				7484.85
216	mGpsmCpsmUpsmCpsmCpsmApsApsApsTpsTpsCpsTpsTpsTpsApsmUpsmApsmApsmGpsmGpsmG/3CholTEG/				7829.72

* Log Reduction post 3x30mg/kg SC

Table 12

#ID	Sequence (5'-3')	2215 HBsAg EC50 (nM)	2215 CC50 (nM)	MW
217	5'-mGps5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGp5mmCpsmGpsmApsmApsmGpsmUpsmGpsm5meC-3	A	I	7009.74
218	5'-mGps5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5mmCpsmGpsmApsmApsmGpsmUpsmGps5mmC-Cholesterol-3'	B	I	7764.7
219	5'-mGps5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGp5mmCpsmGpsmApsmApsmGpsmUpsmGps5mmC-TEG-Cholesterol-3'	B	I	7977.84

220	5'-mGps5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5mmCpsmGpsmApsmApsmGpsmUpsmGps5mmC-Tocopherol-3'	B	I	7708.65
221	5'-mGps5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5mmCpsmGpsmApsmApsmGpsmUpsmGps5mmC-TEG-Tocopherol-3'			7920.79
222	5'-mGps5mmCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5mmCpsmGpsmApsmApsmGpsmUpsmGps5mmC-GalNAc-3'	B	I	8770.65

Table 13

#ID	Sequence (5'-3')	2215 HBsAg EC50 (nM)	2215 CC50 (nM)	MW
223	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsmGpsmApsmApsmGpsmUpsmGpsm5meC-3'	A	I	6979.71
224	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsmGpsmApsmApsmGpsmUpsmGpsm5meC-po-Chol-3'		I	7735.67
225	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsmGpsmApsmApsmGpsmUpsmGpsm5meC-po-Tocopherol-3'		I	7678.62
226	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsmGpsmApsmApsmGpsmUpsmGpsm5meC-po-GalNAc-3'	B	I	8740.62
227	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsGpsmApsmApsmGpsmUpsmGpsm5meC-3'	A	I	6949.69
228	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsGpsmApsmApsmGpsmUpsmGpsm5meC-po-Chol-3'		I	7705.65
229	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsGpsmApsmApsmGpsmUpsmGpsm5meC-po-Tocopherol-3'	A	I	7650.61
230	5'-mGpsm5meCpsmApsmGpsmApsmGpsGpsTpsGpsApsApsGps5meCpsGpsmApsmApsmGpsmUpsmGpsm5meC-po-GalNAc-3'	B	I	8710.59

Table 14

#ID	Sequence (5'-3')	2215 HBsAg EC50 (nM)	2215 HBsAg CC50 (nM)	MW
231	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps (5m)mC-3	A	I	6967.62
232	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps (5m)mC-Chol-3	A	I	7723.58
233	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps (5m)mC-Toco-3	A	I	7666.53
234	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps (5m)mC-GalNAc-3	A	I	8728.52
235	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps (5m)mC-3	A	I	6937.59
236	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps (5m)mC-Chol-3	A	I	7693.55
237	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps (5m)mC-Toco-3	A	I	7636.50
238	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps (5m)mC-GalNAc-3	A	I	8698.50
239	5-mGps2-4-OCH_2-(5m)CpsmAps 2-4-OCH_2-GpsmAps2-4-OCH_2-GpsGpsTpsGpsApsApsGps(5m)CpsmGps2-4-OCH_2-ApsmAps2-4-OCH_2-GpsmUps2-4-OCH_2-Gps (5m)mC-3	A	I	6881.57

Table 15

#ID	Sequence (5'-3')	2215 HBsAg EC 50 (nM)	2215 CC50 (nM)	MW

240	5-dTnpsGnpsCnpsAnpsGnpsApsGpsGpsTpsGpsApsApsGpsCpsGpsAnpsAnpsGnpsTnpsGn-3	A	I	6566.49
241	5-dTnpsfGnpsCnpsfAnpsfGnpsApsGpsGpsTpsGpsApsApsGpsCpsGpsfAnpsfAnpsfGnpsfUnpsfGn 3'	A	I	6700.35

Table 16

#ID	Sequence (5'-3')	2215 HBsAg EC50	2215 CC50	MW
243	5-fGnpsCnpsfAnpsfGnpsfAnpsfGnpsGpsTpsGpsApsApsGpsCnpsfGnpsfAnpsfAnpsfGnpsfUnpsfGnpsCn-3'	A	I	6731.41
242	5-fGnpsCnpsfAnpsfGnpsfAnpsfGnpsGpsTpsGpsApsApsGpsCpsGpsfAnpsfAnpsfGnpsfUnpsfGnpsCn-3'	A	I	6715.39

Table 17

#ID	Y Domain Size	Sequence (5'-3')	2215 EC 50	2215 CC50 (nM)	MW
244	6	5'-dGnpmCnpmAnpmGnpmAnpmGnpGpsTpsGpsApsApsGpsmCnpmGnpmAnpmAnpmGnpmUnpmGnpmCnp-3'	A	I	6714.98
245	7	5'-dGnpmCnpmAnpmGnpmAnpmGnpGpsTpsGpsApsApsGpsCpsmGnpmAnpmAnpmGnpmUnpmGnpmCnp-3'	A	I	6702.01
246	8	5'-dGnpmCnpmAnpmGnpmAnpmGnpGpsTpsGpsApsApsGpsCpsGpsmAnpsmAnpmGnpmUnpmGnpmCnp-3'	A	G	6689.03
247	8	5'-dGnpmCnpmAnpmGnpmAnpGpsGpsTpsGpsApsApsGpsCpsmGnpmAnpmAnpmGnpmUnpmGnpmCnp-3'	A	I	6689.03
248	9	5'-dGnpmCnpmAnpmGnpmAnpGpsGpsTpsGpsApsApsGpsCpsGpsmAnpmAnpmGnpmUnpmGnpmCnp-3'	A	I	6676.06

Table 18

#ID	Sequence (5'-3')	2215 HBsAg EC50	2215 HBsAg CC50	MW

142	5'-mApsmApsmGpsmApsmGpsApsGpsGpsTpsGps5meCps Gps5meCps5meCps5meCps5memCpsmGpsmUpsmGpsmG 3'	A	I	6869.64
196	5'-mApsmApsmGpsmApsmGpsmApsGpsGpsTpsGps5meC psGps5meCps5meCps5memCps5memCpsmGpsmUpsmGps mG 3'	A	I	6929.69
197	5'-mApsmApsmGpsmApsmGpsmApsmGpsGpsTpsGps5me CpsGps5meCps5memCps5memCps5memCpsmGpsmUpsm GpsmG 3'	A	I	6989.74
143	5'-mGpsmGpsmUpsmGpsmApsApsGps5meCpsGpsApsAps GpsTpsGps5mCpsmAps5memCpsmAps5memeCpsmG 3'	A	I	6863.64
198	5'-mGpsmGpsmUpsmGpsmApsmApsGps5meCpsGpsApsA psGpsTpsGps5memCpsmAps5memCpsmAps5memeCpsm G 3'	A	I	6923.69
199	5'-mGpsmGpsmUpsmGpsmApsmApsmGps5meCpsGpsAps ApsGpsTpsGps5memCpsmAps5memCpsmAps5memeCps mG 3'	A	I	6953.72
146	5'-mUpsmGpsmGps5memCpsmAps5meCpsTpsApsGpsTps ApsApsAps5meCpsTpsmGpsmApsmGps5memCps5memC 3'	A	I	6987.62
200	5'-mUpsmGpsmGps5memCpsmAps5memCpsTpsApsGpsT psApsApsAps5meCpsTpsmGpsmApsmGps5memCps5mem C 3'	A	I	6817.64
201	5'-mUpsmGpsmGps5memCpsmAps5meCpsTpsApsGpsTps ApsApsAps5meCpsTpsmGpsmApsmGps5memCps5memC 3'	A	I	
147	5'-5memCpsmUpsmApsmGmGpsApsGpsTpsTps5meCps5 meCpsGps5meCpsApsGpsmUpsmApsmUpsmGpsmG 3'	A	I	6792.55
202	5'-5memCpsmUpsmApsmGmGpsmApsGpsTpsTps5meCps 5meCpsGps5meCpsApsmGpsmUpsmApsmUpsmGpsmG 3'	A	I	6852.60
203	5'-5memCpsmUpsmApsmGmGpsmApsmGpsTpsTps5meC ps5meCpsGps5meCpsApsmGpsmUpsmApsmUpsmGpsmG 3'	A	I	6882.62

Table 19

#ID	Sequence (5'-3')	2215 HBsAg EC50	2215 CC50	MW
128	5'-GnpsCnpsAnpsGnpsApsGpsGpsTpsGpsAnpsAnpsGnpsC nps-3-NH_2-G-3'	B	J	4577.86

129	5'-CnpsGnpsTnpsGnpsCnpsApsGpsApsGpsGpsTnpsGnpsAnpsAnpsGnps-3-NH_2-C-3'	B	J	5201.40
130	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGpsApsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'	A	J	6543.60
131	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTpsGpsAnpsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'	B	J	6543.60
132	5'-GnpsCnpsAnpsGnpsAnpsGnpsGpsTpsGnpsAnpsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'	B	J	6543.60
133	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGnpsApsApsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'	B	J	6543.60
134	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGnpsAnpsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'	B	J	6543.60
135	5'-GnpsCnpsAnpsGnpsAnpsGnpsGpsTpsGpsAnpsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'	B	J	6544.58
136	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGpsApsApsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'	B	J	6544.58
137	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTpsGpsApsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'	A	J	6544.58
138	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGnpsApsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'	B	J	6544.58
139	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGpsApsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'	A	J	6545.57
140	5'-GnpsCnpsAnpsGnpsAnpsGnpsGnpsTpsGpsApsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3 NH_2-C-3'	A	J	6546.55

Table 20

#ID	2215 HBsAg EC50 (nM)	2215 CC50 (nM)	2217 sup HBVDNA (EC50 nM)	2117 intra HBVNA EC50(nM)
177	B	J	D	F
178	B	J	E	F
179	B	J	F	F
180	A	J	D	F
181	B	J	D	F
182	A	J	D	E
183	A	J	D	E
184	A	J	D	D
185	A	J	D	E
186	B	J	D	D
187	B	J	E	E
188	B	J	E	F
189	A	J	D	F

190	C	J	E	F
191	B	J	E	F
192	B	J	D	D
193	B	J	E	D
194	C	J	E	E
195	B	J	E	D

Table 21

#ID	2215 HBsAg EC50 (nM)	2215 CC50 (nM)	2217 sup HBVDNA (EC50 nM)	2217 intra HBVDNA (EC50 nM)
124	B	J	B	B
125	B	J	B	B
126		J	B	A
127	A	J	A	A
128	B	J	C	B
129	B	J	B	B

Table 22

	2215 HBsAg EC50 (nM)	2215 CC50 (nM)	2217 sup HBVDNA (EC50 nM)	2217 intra HBVDNA (EC50 nM)
249	C	G	F	F
250	B	G	F	E
251	B	G	F	F
252	B	G	E	E
253	B	G	E	E
254		G	E	D

255	A	G	D	D
256	B	G	F	E
257	B	G	E	E

Table 23

HBsAg 2215 EC50 (μM)	2215 CC50 (μM)	Sequence (5'-3')	MW
A	E-F	GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGpsApsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3nh2-C	6543.60
A	E-F	GnpsCnpsAnpsGnpsAnpsGnpsGnpsTpsGpsAnpsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3nh2-C	6543.60
A	E-F	GnpsCnpsAnpsGnpsAnpsGnpsGpsTpsGnpsAnpsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3nh2-C	6543.60
A	E-F	GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGnpsApsApsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3nh2-C	6543.60
A	E-F	GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGnpsAnpsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3nh2-C	6543.60
A	E-F	GnpsCnpsAnpsGnpsAnpsGnpsGpsTpsGpsAnpsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3nh2-C	6544.58
A	E-F	GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGpsApsApsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3nh2-C	6544.58
A	E-F	GnpsCnpsAnpsGnpsAnpsGnpsGnpsTpsGpsApsAnpsGnpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3nh2-C	6544.58
A	E-F	GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGnpsApsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3nh2-C	6544.58
A	E-F	GnpsCnpsAnpsGnpsAnpsGnpsGnpsTnpsGpsApsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3nh2-C	6545.57
A	E-F	GnpsCnpsAnpsGnpsAnpsGnpsGnpsTpsGpsApsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3nh2-C	6546.55
A	E-F	GnpsCnpsAnpsGnpsAnpsGnpsGpsTpsGpsApsApsGpsCpsGnpsAnpsAnpsGnpsTnpsGnps-3nh2-C	6548.52
A	E-F	GnpsCnpsApsGnpsAnpsGpsGnpsTnpsGpsAnpsAnpsGpsCnpsGnpsApsAnpsGnpsTpsGnps-3nh2-C	6547.54
A	E-F	GpsCnpsAnpsGpsAnpsGnpsGpsTnpsGnpsApsAnpsGnpsCpsGnpsAnpsApsGnpsTnpsGps-3nh2-C	6548.52
A	E-F	GnpsCpsAnpsGnpsApsGnpsGnpsTpsGnpsAnpsApsGnpsCnpsGpsAnpsAnpsGpsTnpsGnps-3nh2-C	6547.54

A	E-F	GnpsCnpsAnpsGnpsAnpsGnpsGpsTpsGpsApsApsGpsCnpsGnpsAnpsAnpsGnpsTnpsGnps-3nh2-C	6547.54

[0325] Two oligonucleotides, the first containing 2'MOE PS modifications and the other containing 2'MOE NPS, were tested in vitro and in vivo. The following Tables 24-26 summarize the results of the testing.

Table 24

Sequence	T_m (°C)	Max HBsAg Reduction (nadir) 3x10 mg/kg	Max HBeAg Reduction (nadir) 3x10 mg/kg	
258	77.2	3.4 log	2.7 log	
259	69.9	2.4 log	1.9 log	
Improvement	7.3	1 log	0.8 log	
#	Sequence (5'-3')			Mol Wt.
258	5'-mGnpsmoeCnpsmoeAnpsmGnpsmoeAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmoeAnpsmGnpsmoeUnpsmGnpsmoeCnp-C6-NH-GalNAc6-3'			8862.97
259*	5'-moeGps(5me)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsApsGps(5me)CpsGpsApsmoeApsmoeGpsmoeTpsmoeGps(5me)moeC-po-GalNAc2-3'			9008.93

*Sequences 260 and 261 were also tested and provided similar results.

[0326] FIG. 9(a) shows HBsAg results of oligomers 1 and 2 in a HBV Mouse Model tested at 3x 10 mg/kg on Days 0, 2, 4. Figure 9(b) shows the HBeAg results.

Table 25

Sequence	T_m (°C)	Max HBsAg Reduction (nadir) 3x10 mg/kg	Max HBeAg Reduction (nadir) 3x10 mg/kg	
262	77.3	3.1 log	2.5 log	
263	69.9	2.4 log	1.9 log	
Improvement	7.4	0.7 log	0.6 log	
#	Sequence (5'-3')			Mol Wt.

262*	5'-GalNAc 2-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn 3'	8941.00
263	5'-moeGps(5me)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsApsGps(5me)CpsGpsApsmoeApsmoeGpsmoeTpsmoeGps(5me)moeC-GalNAc2-3'	9008.93

*5'-GalNac2-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn-3'
and
5'-GalNac-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn-3' were also tested and provided similar results.

Table 26

Sequence	Max HBsAg Reduction (nadir) 3x5 mg/kg	Max HBeAg Reduction (nadir) 3x5 mg/kg
266	2.3 log	2.1 log
267	2.2 log	1.9 log
Improvement	0.1 log	0.2 log

#	Sequence (5'-3')	Mol Wt.
266*	5-GalNAc2-mGnpsmCnpsmAnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn-3	8736.73
267	5'-GalNac6- NH-C6-moeGps(5m)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeApsmoeGpsmoeTpsmoeGps(5m)moeC-3'	9105.14

*5'-GalNAc2-mGnpsmCnpsmAnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmAnpsmGnpsmUnpsmGnpsmCn-3' was also tested and provided similar results.

[0327] As can be seen above, the MOE NPS oligomers were more active than MOE PS *in vivo* and OMe NPS is as active as MOE PS oligomers.

[0328] Two oligonucleotides, the first containing OEt NPS substitution and the second having MOE NPS were tested in vitro and in vivo. The following Table 27 summarizes the results of the testing.

Table 27

Sequence	Max HBsAg Reduction (nadir) 3x5 mg/kg	Max HBeAg Reduction (nadir) 3x5 mg/kg
269	1.9 log	1.7 log
270	1.9 log	1.8
Difference	0 log	-0.1 log

#	Sequence (5'-3')
269	5'-GalNAc2-etoGnps(5m)etoCnpsetoAnpsetoGnpsetoAnpsGps GpsTpsGpsApsApsGps(5m)CpsGpsApsetoAnpsetoGnpsetoTnpsetoGnps(5m)etoCn-3'
270	5-GalNAc2-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmAnpsmGnpsmUnpsmGnpsmCn-3

[0329] As can be seen above, the MOE NPS oligomers had similar activity to the OEt NPS oligomers.

[0330] Four oligonucleotides, the first containing MOE PS substitution, the second having MOE NPS substitution, the third having OME PS substitution, the fourth having OME NPS were tested in vitro. The following Table 28 summarizes the results of the testing. Comparing with Sequence #9 (MOE PS), Sequence #10 (MOE NPS) is 7 times more potent in vitro. Comparing with Sequence #11 (OME PS), Sequence #12 (OME NPS) is close to 6 times more potent.

Table 28

Sequence	2215 HBsAg EC50 (nM)	T_m (°C)
271	5	69.9
272	0.7	77.3
273	5	70.7
274	0.9	75.5

#	Sequence (5'-3')	MW

271	5'-moeGpsmoemCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsAps Gps5mCpsGpsApsmoeApsmoeGpsmoeTpsmoeGpsmoemC 3'	7344.19
272	5'moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsA psApsGpsCpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn 3'	7276.27
273	5'-mGps5mmCpsmApsmGpsmApsGpsGpsTpsGpsApsApsGps5mCps GpsApsmApsmGpsmUpsmGps5mmC-3'	6889.64
274	5'-mGnpsmCnpsmAnpsmGnps mAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmAnpsmGnpsmUnp smGnpsmCn-3'	6837.71

[0331] Two oligonucleotides, the first containing 5'GalNAc-2'-MOE NPS substitution, the second having 5'-GalNAc-6: MOE PS substitution was tested in vivo. The following Table 29, along with FIG. 8, summarizes the results of the testing. Maximum HBsAg reduction (nadir) improvement is shown in Table 29. At certain times the advantage was as high as 0.8 log (6x) difference and the advantage of MOE NPS over MOE PS was maintained throughout most days of 42-day study duration.

Table 29

#	Sequence (5'-3')	MW
275	5'-GalNAc2-moeGnpsmoeCnpsmoeAnps moeGnpsmoeAnpsGps GpsTpsGps ApsApsGps (5m)CpsGpsAps moeAnpsmoeGnpsmoeUnps moeGnpsmoeCn-3'	8957.00
276	5'-GalNac6-NH-C6-moeGps(5m)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsA psGps(5m)CpsGpsApsmoeApsmoeGpsmoeTpsmoeGps(5m)moeC-3'	9105.14

Dose	Improvement of HBsAg Max Reduction (nadir)
3x5 mg/kg	0.4 Log (2.5 times)
1x5 mg/kg	0.5 log (3.2 times)

[0332] Two oligonucleotides, the first containing 3'-GalNAc-2'-MOE NPS substitution, the second having 3'-GalNAc2'-MOE PS substitution was tested in vivo. The following Table 30 summarizes the results of the testing.

Table 30

#	Sequence (5'-3')	MW
277	5'moeGps(5m)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsAps Gps(5m)CpsGpsApsmoeApsmoeGpsmoeTpsmoeGps(5me)moeC-GalNAc2-3'	9008.93
258	5'-mGnpsmoeCnpsmoeAnpsmGnpsmoeAnpsGpsGpsTpsGpsApsApsGpsC psGpsApsmoeAnpsmGnpsmoeUnpsmGnpsmoeCnp-C6-NH-GalNAc6 3'	8862.97

Sequence	277	258	Improvement
T_m (°C)	69.9	77.2	7.3
2215 HBsAg In vitro EC50 (nM)	5	0.7	7.1-fold
Max HBsAg Reduction (nadir)3x10 mg/kg	2.4 log	3.4 log	1 log (10 times)
Max HBeAg Reduction (nadir) 3x10 mg/kg	1.9 log	2.7 log	0.8 log (6.3 times)

[0333] Two oligonucleotides, the first containing OME NPS substitution, the second having OME PS substitution were tested in vivo. The following Table 31, along with FIG. 10, summarizes the results of the testing. OME NPS is much more potent in vivo than OME PS.

Table 31

#	Sequence (5'-3')	MW
278	5-GalNAc2-mGnpsmCnpsmAnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsA psmAnpsmGnpsmUnpsmGnpsmCn-3'	8502.45
279	5-mGps(5m)mCpsmApsmGpsmApsGpsGpsTpsGpsApsApsGps(5m)CpsG psApsmApsmGpsmUpsmGps(5m)mC-GalNAc-3'	8650.54

Improvement of OME NPS over OME PS	Max HBsAg Reduction (nadir) improvement	Max HBeAg Reduction (nadir)
3x10 mg/kg	0.9 Log (8 times)	0.5 Log (3.2 times)

[0334] The following sequences were tested in the HBV mouse model. The results are shown in FIG. 11. In FIG. 11A, at 1x10mg/kg dose, 3' GalNac MOE NPS maintained as high as 0.8 log (6 times) better efficacy than 5' GalNac MOE PS, advantage was maintained throughout most of the 21 day study. 5'GalNac MOE NPS maintained as high as 0.4 log (2.5 times) better efficacy than 5' GalNac MOE PS, advantage was maintained throughout most of the 21 day study. In FIG. 11B, at 3x3.3 mg/kg dose, 3' GalNac MOE NPS and 5'GalNac MOE NPS performed similarily, both maintained as high as 0.6 log (4 times) better efficacy than 5' GalNac MOE PS, advantages were maintained throughout most of the 21 day study.

Table 32

#	Chemistry	Sequence (5' – 3')	MW
276	MOE PS	5'-GalNac6-NH-C6-moeGps(5m)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeApsmoeGpsmoeTpsmoeGps(5m)moeC-3'	9105.14
280	MOE NPS	5'-GalNAc2-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn-3'	8957.00

[0335] The following sequences were tested in the HBV mouse model. The results are shown in FIG. 11A for a dosing regimen of a 10 mg/kg single dose, and FIG. 11B for a dosing regimen of 3x3.3 mg/kg on Days 0, 2, 4.

Table 33

#	Chemistry	Sequence (5' – 3')	MW
276	MOE PS	5'-GalNac6-NH-C6-moeGps(5m)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeApsmoeGpsm	9105.14

		oeTpsmoeGps(5m)moeC-3'	
281	MOE NPS	5'-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCnp-C6-NH-GalNAc6-3'	9053.85
282	MOE NPS	5'-GalNAc2-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnps moeGnpsmoeCn-3'	8957.00

[0336] The following sequences were tested in the HBV mouse model. The values in the right column show max HBsAg reduction in LOG dosed at 3x10 mg/kg Days 0, 2, 4.

Table 34

#	Chemistry	Sequence 5'-3'	Max HBsAg reduction (nadir)	MW
283	Deoxy NPS	5'-GalNAc-GnpsCnpsAnpsGnpsAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsAnpsGnpsTnpsGnpsCn-3'	1.1	8312.38
265	MOE NPS	5'-GalNAc-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn-3'	3.1	9037.17

[0337] The following sequences were tested in the HBV mouse model. The values in the right column show max HBsAg reduction in LOG dosed at 3x10 mg/kg Days 0, 2, 4.

Table 35

No.	Targeted HBV Region	Chemistry	Sequence 5'-3'	Max HBsAg reduction (nadir) in log	MW
283	DR2 #1	Deoxy NPS	5'-GalNAc-	1.1	8312.

			GnpsCnpsAnpsGnpsAnpsGps GpsTpsGpsApsApsGpsCpsGpsAps AnpsGnpsTnpsGnpsCn-3'		38
284	DR2 #1	OME NPS	5'-GalNAc-mGnpsmCnpsmAnpsmGnpsmAnps Gps GpsTpsGpsApsApsGpsCpsGpsAps mAnpsmGnpsmUnpsmGnpsmCn-3'	2.1	8598.62
285	DR2 #1	F NPS	5'-GalNAc-fGnpsfCnpsfAnpsfGnpsfAnpsGpsG psTpsGpsApsApsGpsCpsGpsApsfA npsfGnpsfUnpsfGnps-3nh2-fC-3'	2.5	8478.26
286	DR2 #1	Ara F NPS	5'-GalNAc-afGnpsafCnpsafAnpsafGnpsafAnps GpsGpsTpsGpsApsApsGpsCpsGps ApsafAnpsafGnpsafTnpsafGnpsafC n-3'	0.5	8492.29
287	DR2 #2	Deoxy NPS	5'-GalNAc-dTnpsGnpsCnpsAnpsGnpsApsGpsG psTpsGpsApsApsGpsCpsGpsAnpsA npsGnpsTnps-3nh2-G-3'	1.1	8327.29
288	DR2 #2	OME NPS	5'-GalNAc-mUnpsmGnpsmCnpsmAnpsmGnps ApsGpsGpsTpsGpsApsApsGpsCps GpsmAnpsmAnpsmGnpsmUnpsmG n-3'	2.1	8599.60
289	DR2 #2	F NPS	5'-GalNAc-fUnpsfGnpsfCnpsfAnpsfGnpsApsG psGpsTpsGpsApsApsGpsCpsGpsfA npsfAnpsfGnpsfUnps-3nh2-fG-3'	2.4	8479.24
290	Pre-PolyA	OME NPS	5'-GalNAc-mGnpsmCnpsmUnpsmCnpsmCnps ApsApsApsTpsTps5MeCpsTpsTpsT psApsmUnpsmAnpsmAnpsmGnpsm GnpsmGn-3'	1.1	8807.84
291	Pre-PolyA	MOE NPS	5'-GalNAc-moeGnpsmoeCnpsmoeUnpsmoeCnp smoeCnpsApsApsApsTpsTps5MeC psTpsTpsTpsApsmoeUnpsmoeAnps	2.0	9292.42

			moeAnpsmoeGnpsmoeGnpsmoeGn-3’		

[0338] The following oligomers having MOE/NPS and MOE/PS substitution were tested using (1) a HepG2.2.15 HBsAg reduction potency comparison, (2) a HepG2.117 HBV DNA reduction potency comparison, (3) a Primary Human Hepatocyte (PHH) HBsAg reduction potency comparison, (4) a Primary Human Hepatocyte (PHH) HBeAg reduction potency comparison.

Table 36

		1	2	3	4	
No.	Sequence (5’-3’)	2215 HBsAg EC50 (nM)	2117 HBV DNA EC50 (nM)	PHH HBsAg EC50 (nM)	PHH HBeAg EC50 (nM)	MW
292	5’-moeGps(5m)moeCpsmoeApsmoeGpsmoeApsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeApsmoeGpsmoeTpsmoeGps(5m)moeC-3’	5.1	11.4	16.2	20.1	7344.19
293	5’-moeGnpsmoeCnpsmoeAnpsmoeGnpsmoeAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsmoeAnpsmoeGnpsmoeUnpsmoeGnpsmoeCn-3’	0.43	1.9	1.7	2.5	7292.26

Table 37

No.	Chemistry	MW	Sequence
294	F NPS with OPO link to 3’GalNac	8507.3	5’-fGnps(5m)fCnpsfAnpsfGnpsfAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsfAnpsfGnpsfTnpsfGnpsfC-C6-NH-GalNac6-3’

295	F NPS with NPO link to 3'GalNac	8492.29	5'-fGnpsfCnpsfAnpsfGnpsfAnpsGpsGpsTpsGpsApsApsGps(5m)CpsGpsApsfAnpsfGnpsfUnpsfGnpsfCnp-C6-NH-GalNAc6-3'

[0339] As shown in FIG. 12A, at 1x 10 mg/kg, F NPS with OPO linkage to 3'GalNac significantly outperformed F NPS with NPO linkage, as high as 1.2 log (16 times) better at certain time points.

Table 38

No.	Chemistry	MW	Sequence
296	OME NPS with NPO linkage to 3'GalNac	8614.39	5-mGnpsmCnpsmAnps mGnpsmAnpsGps GpsTpsGps ApsApsGps (5m)CpsGpsAps mAnpsmGnpsmUnps mGnpsmCnp-C6-NH-GalNAc6-3
297	OME NPS with OPO linkage to 3'GalNac	8614.43	5-mGnpsmCnpsmAnps mGnpsmAnpsGps GpsTpsGps ApsApsGps (5m)CpsGpsAps mAnpsmGnpsmUnps mGnpsmC-C6-NH-GalNAc6-3

[0340] As shown in FIG. 12B, at 1x 10 mg/kg, OME NPS with OPO linkage to 3'GalNac significantly outperformed OME NPS with NPO linkage, as high as 0.7 log (5 times) better at certain time points.

Table 39

No.	Chemistry	MW	Sequence
298	MOE NPS with NPO linkage to 3'GalNac	9053.85	5'-moeGnpsmoeCnpsmoeAnps moeGnpsmoeAnpsGps GpsTpsGps ApsApsGps (5m)CpsGpsAps moeAnpsmoeGnpsmoeUnps moeGnpsmoeCnp-C6-NH-GalNAc6-3'
299	MOE NPS with OPO linkage to 3'GalNac	9069.62	5'-moeGnpsmoeCnpsmoeAnps moeGnpsmoeAnpsGps GpsTpsGps ApsApsGps (5m)CpsGpsAps moeAnpsmoeGnpsmoeUnps moeGnps(5m)moeC-C6-NH-GalNAc6-3'

Table 40

No.	Chemistry	MW	Sequence

300	5' GalNac MOE NPS	8955.48	5-GalNAc2-moeGnpsmoeCnpsmoeAnps moeGnpsmoeAnpsGps GpsTpsGps ApsApsGps (5m)CpsGpsAps moeAnpsmoeGnpsmoeUnps moeGnpsmoeCn-3
301	5' GalNac OEt NPS	8697.6	5-GalNAc2-etoGnpseto(5m)CnpsetoAnps etoGnpsetoAnpsGps GpsTpsGps ApsApsGps (5m)CpsGpsAps etoAnpsetoGnpsetoTnps etoGnpseto(5m)Cn-3'

[0341] As shown in FIG. 12C, at 1x 10 mg/kg, OEt NPS is as efficacious as MOE NPS.

Table 41

No.	Sequence 5'-3'	Modification	MW	2215 HBsAg EC50 (uM)	2215 HBsAg CC50 (uM)
302	5-mGnpsmCnps2-4-OCH_2AnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsAps2-4-OCH_2AnpsmGnpsmUnpsmGnps3-NH_2mC-3	Anti-DR-1 with x2 3'-NH-LNA-A	6835.3	0.0008	0.0148
303	5-mGnpsmCnps2-4-OCH_2CH_2AnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsAps2-4-OCH_2CH_2AnpsmGnpsmUnpsmGnps3-NH_2mC-3	Anti-DR-1 with x2 3'-NH-ENA-A	6862.0	0.00067	0.0256
304	5-mGnpsmCnps2-4-OCH_2CH_2AnpsmGnps2-4OCH_2CH_2AnpsGpsGpsTpsGpsApsApsGpsCpsGpsAps2-4-OCH_2CH_2AnpsmGnpsmUnpsmGnps3-NH_2mC-3	Anti-DR-1 with x3 3'-NH-ENA-A	6874.7	0.0009	0.0214
305	5-mGnpsmCnpsmAnpsmGnps2-4-OCH_2CH_2AnpsGpsGpsTpsGpsApsApsGpsCpsGpsAps2-4-OCH_2CH_2AnpsmGnpsmUnpsmGnps3-NH_2mC-3	Anti-DR-1 with x2 3'-NH-ENA-A	6863.3	0.00029	0.0226

306	5-mGnpsmCnpsmUnpsmCnpsmCnps2-4-OCH_2CH_2AnpsApsApsTpsTpsCpsTpsTpsTps mAnpsmUnpsmAnps2-4-OCH_2CH_2AnpsmGnpsmGnps3-NH_2mG-3	Pre Poly A with x2 3'-NH-ENA-A	7116.0	0.0005	>1.00
307	5-mGnpsmCnpsmUnpsmCnpsmCnps2-4-OCH_2CH_2AnpsApsApsTpsTpsCpsTpsTpsTps2-4-OCH_2CH_2AnpsmUnpsmAnps2-4-OCH_2 CH_2AnpsmGnpsmGnps3-NH_2mG-3	Pre Poly A with x3 3'-NH-ENA-A	7128.6	0.00055	>1.00
308	5-mGnpsmCnpsmUnpsmCnpsmCnps2-4-OCH_2CH_2AnpsApsApsTpsTpsCpsTpsTpsTps2-4-OCH_2 CH_2AnpsmUnps2-4-OCH_2CH_2AnpsmAnpsmGnpsmGnps3-NH_2mG-3	Pre Poly A with x3 3'-NH-ENA-A	7127.9	0.0006	>1.00

Table 42

No.	Oligonucleotides (5'-3')	Modification	2215 HBsAg EC50 (uM)	2215 HBsAg CC50 (uM)
XX	5-mGnpsmCnpsmAnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmAnpsmGnpsmUnpsmGnps3-NH_2mC-3	Control	---	---
309	5-2-4 OCH_2CH_2GnpsmCnpsmAnpsmGnpsmAnpsGpsGpsTpsGpsApsApsGpsCpsGpsApsmAnpsmGnpsmUnps2-4 OCH_2CH_2Gnps3-NH_2mC-3	DR-1 with 3'-NH-ENA-G(1+1)	0.0013	0.0553

310	5-2-4 OCH_2CH_2GnpsmCnpsmAnps2-4 OCH_2CH_2GnpsmAnpsGpsGpsTpsGpsAp sApsGpsCpsGpsApsmAnps2-4 OCH_2CH_2GnpsmUnps2-4 OCH_2CH_2Gnps3-NH_2mC-3	DR-1 with 3'-NH-ENA-G 2+2 3'-NH-ENA-G	0.0006	0.0230
311	5-2-4 OCH_2CH_2GnpsmCnps2-4- OCH_2CH_2AnpsmGnpsmAnpsGpsGpsTp sGpsApsApsGpsCpsGpsApsmAnpsmGn psmUnpsmGnps3-NH_2mC-3	DR-1 with 3'-ENA-G & 3'-ENA A (1+1) Asymmetric	0.00078	0.0305
312	5-2-4 OCH_2CH_2GnpsmCnpsmUnpsmCnpsmC nps2-4- OCH_2CH_2AnpsApsApsTpsTpsCpsTpsTp sTps mAnpsmUnpsmAnps2-4- OCH_2CH_2AnpsmGnpsm2-4 OCH_2CH_2Gnps3-NH_2mG-3	Pre Poly A with 1+1/1+1 3'—NH-ENA-G+A	0.0015	>1.00
313	5-2-4 OCH_2CH_2GnpsmCnpsmUnpsmCnpsmC npsmAnpsApsApsTpsTpsCpsTpsTpsTps mAnpsmUnpsmAnpsmAnpsmGnpsm2-4 OCH_2CH_2Gnps3-NH_2mG-3	Pre Poly A with 3'-NH-ENA-G 1+1	0.0017	>1.00

Table 43

No.	Found MW:	Oligonucleotides (5'-3')	Modification	HPLC Purity	2215 HBsAg EC50 (uM)	2215 HBsAg CC50 (uM)
314	6838.8	5-mGnpsmCnpsmAnpsmGnpsmAnpsGps GpsTpsGpsApsApsGpsCpsGpsApsmAn psmGnpsmUnpsmGnps3-NH_2mC-3	Control	86%	---	---
315	6902.9	5-mGnps2-4 OCH_2CH_2 (5me)CnpsmAnps2-4 OCH_2CH_2GnpsmAnpsGpsGpsTpsGpsA psApsGpsCpsGpsApsmAnpsmGnps2-4 OCH_2CH_2TnpsmGnps3- NH_2mC-3	DR-1 2+1	83%	0.003 3	>1.00
316	6914.8	5-2-4 OCH_2CH_2GnpsmCnps2-4 OCH_2CH_2AnpsmGnpsmAnpsGpsGpsTp sGpsApsApsGpsCpsGpsApsmAnpsmG nps2-4 OCH_2CH_2TnpsmGnps2- $OCH_2CH_2$3-NH_2 (5me)C-3	DR-1 2+2	94%	0.004 3 0.002 5	>1.00

317	7169.0	5-2-4 OCH_2CH_2GnpsmCnps2-4 OCH_2CH_2TnpsmCnpsmCnpsmAnpsAps ApsTpsTpsCpsTpsTpsTpsmAnps2-4 OCH_2CH_2TnpsmAnpsmAnpsmGnps2-4 OCH_2CH_2Gnps3-NH_2mG-3	Pre Poly A 2+2	84%	0.0025	>1.00
318	7182.2	5-mGnps2-4 OCH_2CH_2 (5me)CnpsmUnps2-4 OCH_2CH_2 (5me)CnpsmCnpsmAnpsApsApsTpsTps CpsTpsTpsTpsmAnps2-4 OCH_2CH_2TnpsmAnpsmAnps2-4 OCH_2CH_2GnpsmGnps3-NH_2mG-3	Pre Poly A 2+2	95%	0.0051	>1.00

[0342] In some embodiments, the oligonucleotide of the present disclosure also include an oligonucleotide that is selected from the nucleobase sequence listed in Tables 1-43, independent of the modifications of the sequences listed in Tables 1-43. Oligonucleotides of the present disclosure also include an oligonucleotide comprising a sequence that is at least 90% identical to a nucleobase sequence selected from the sequences listed in Tables 1-43, independent of the modifications of the sequences listed in Tables 1-43. In some embodiments, 1, 2, 3, 4, 5 nucleobases are different from the sequences listed in Tables 1-43, independent of the modifications of the sequences listed in Tables 1-43.

[0343] In some embodiments, the oligonucleotides of the present disclosure also include an oligonucleotide that is selected from the nucleotide sequences listed in Tables 1-43, independent of the nucleobases of the sequences listed in Tables 1-43. Oligonucleotides of the present disclosure also include an oligonucleotide comprising a sequence that is at least 90% identical to a nucleotide sequence selected from the sequences listed in Tables 1-43, independent of the nucleobases of the sequences listed in Tables 1-43. In some embodiments, 1, 2, 3, 4, 5 nucleobases are different from the sequences listed in Tables 1-43, independent of the modifications of the sequences listed in Tables 1-43.

WHAT IS CLAIMED IS:

1. A chimeric antisense oligonucleotide represented by Formula (A):

5' X—Y—Z 3' (A)

wherein:

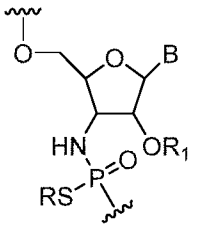
X—Y—Z is a chimeric oligonucleotide comprising a sequence of 18 to 22 nucleosides, optionally conjugated at the 5' and/or 3' end to a ligand targeting group;

X is a domain comprising a sequence of modified nucleosides that is 3-10 nucleosides in length;

Z is a domain comprising a sequence of modified nucleosides that is 3-10 nucleosides in length;

Y is a domain comprising a sequence of 2 to 10 2'-deoxy-nucleosides linked through thiophosphate intersubunit linkages;

each modified nucleoside in the X domain and each modified nucleoside in the Z domain are nucleosides of Formula (1)



(1);

R is H or a positively charged counter ion;

B is a nucleobase;

R_1 is $-(CR'_2)_2OCR'_3$ or –OEt;

R' is independently in each instance H or F; and

said oligonucleotide is complementary to a sequence of the HBV genome.

2. The oligonucleotide of claim 1, wherein:

R is H;

R_1 is $-(CR'_2)_2OCR'_3$; and

each R' is H.

3. The oligonucleotide of claim 1 or claim 2, wherein the X domain and the Z domain each comprise a sequence of modified nucleosides that is 4-6 nucleosides in length.

4. The oligonucleotide of claim 1, wherein R_1 is –OEt.

5. The oligonucleotide of claim 1, wherein R_1 is $-(CH_2)_2OCH_3$.

6. The oligonucleotide of any one of claims 1 to 5, wherein the Y domain sequence comprises 10 nucleosides.

7. The oligonucleotide of any one of claims 1 to 6, wherein the Y domain comprises a nucleobase sequence of GGTGAAG(5m)CGA (SEQ ID NO: 576).

8. The oligonucleotide of any one of claims 1 to 6, wherein the X domain and the Z domain each comprise a sequence of modified nucleosides that is 5 nucleosides in length.

9. The oligonucleotide of any one of claims 1 to 8, wherein the ligand targeting group comprises a GalNAc moiety.

10. A pharmaceutical composition comprising an oligonucleotide of any one of claims 1 to 9 and a pharmaceutically acceptable excipient.

11. A chimeric antisense oligonucleotide represented by Formula (A):

5’ X-Y-Z 3’ (A)

wherein:

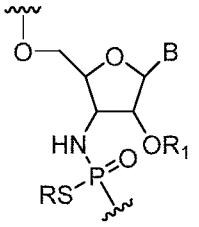
X—Y—Z is a chimeric oligonucleotide comprising a sequence of 18 to 22 nucleosides, optionally conjugated at the 5' and/or 3' end to a ligand targeting group;

X is a domain comprising a sequence of modified nucleosides that is 3-10 nucleosides in length;

Z is a domain comprising a sequence of modified nucleosides that is 3-10 nucleosides in length;

Y is a domain comprising a sequence of 2 to 10 2’-deoxy-nucleosides linked through thiophosphate intersubunit linkages;

each modified nucleoside in the X domain and each modified nucleoside in the Z domain are nucleosides of Formula (1)



(1);

R is H or a positively charged counter ion;

B is a nucleobase;

R_1 is $-CR'_3$, $-CR'_2OCR'_3$, $-(CR'_2)_3OCR'_3$ or $-(CR'_2)_{1-2}CR'_3$,$-(CR'_2)_2OCR'_3$ or –Et;

R' is independently in each instance H or F; and

the chimeric antisense oligonucleotide comprises a nucleobase sequence that is complementary or hybridizes to a target RNA.

12. The oligonucleotide of claim 11, wherein

R is H,

R_1 is $-(CR'_2)_2OCR'_3$, and

each R' is H.

13. The oligonucleotide of claim 11 or claim 12, wherein the X domain and the Z domain each comprise a sequence of modified nucleosides that is 4-6 nucleosides in length.

14. The oligonucleotide of claim 11, wherein R_1 is –Et or $-(CH_2)_2OCH_3$.

15. The oligonucleotide of claim 11, wherein the Y domain sequence comprises 10 nucleosides.

16. The oligonucleotide of claim 15, wherein the X domain and the Z domain each comprise a sequence of modified nucleosides that is 5 nucleosides in length.

17. The oligonucleotide of claim 16, wherein each B is independently selected from the group consisting of adenine, guanine, thymine, cytosine, uracil, 5-methylcytosine, 2,6-diaminopurine, and 5-methyluracil.

18. A pharmaceutical composition comprising an oligonucleotide of any one of claims 11 to 17 and a pharmaceutically acceptable excipient.

19. A method of treating a subject having a viral infection, comprising administering to the subject a therapeutically effective amount of an oligonucleotide of any one of claims 11 to 17 of a pharmaceutical composition of claim 18.

20. Use of an oligonucleotide of any one of claims 11 to 17 of a pharmaceutical composition of claim 18 in the manufacture of a medicament for the treatment of a subject having a viral infection.

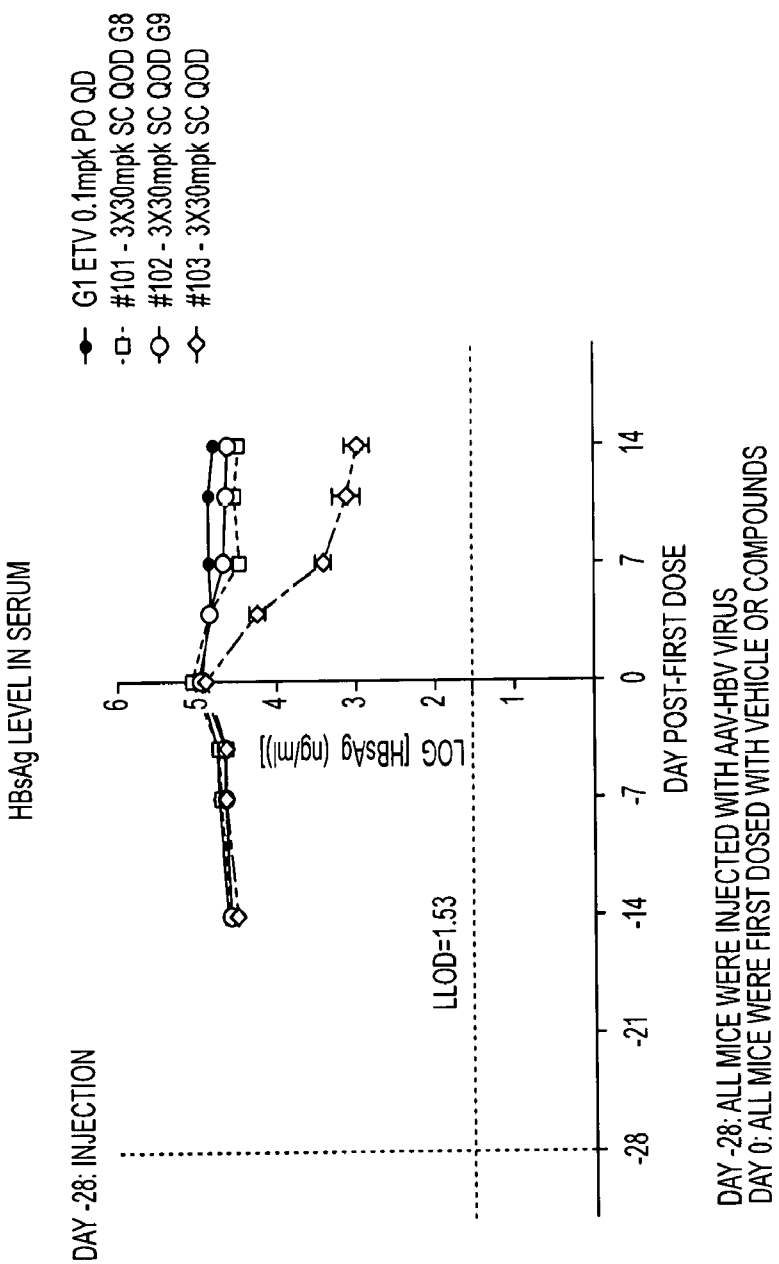

HBsAg LEVEL IN SERUM
G1 ETV 0.1mpk PO QD
#101 - 3X30mpk SC QOD G8
#102 - 3X30mpk SC QOD G9
#103 - 3X30mpk SC QOD
DAY -28: INJECTION
LOG [HBsAg (ng/ml)]
LLOD=1.53
DAY POST-FIRST DOSE
DAY -28: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 1A

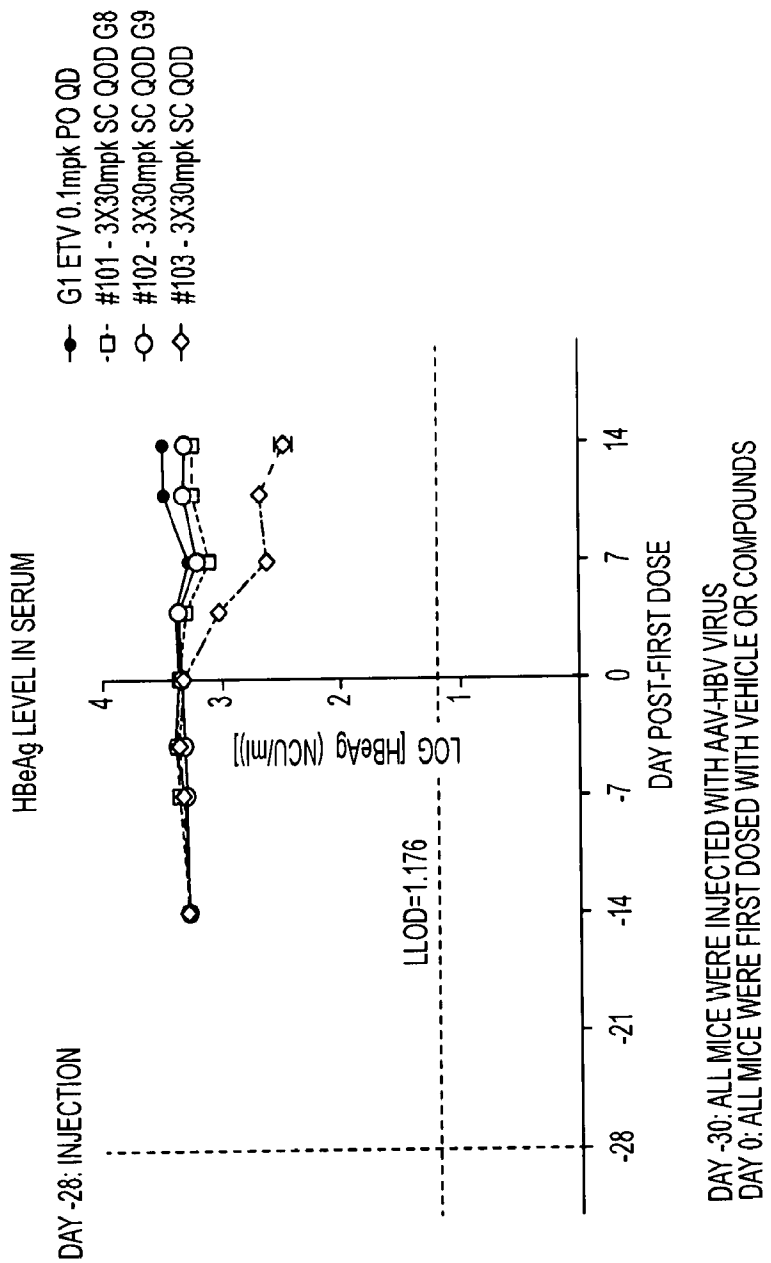

HBeAg LEVEL IN SERUM
G1 ETV 0.1mpk PO QD
#101 - 3X30mpk SC QOD G8
#102 - 3X30mpk SC QOD G9
#103 - 3X30mpk SC QOD
LOG [HBeAg (NCU/ml)]
4
3
2
1
LLOD=1.176
DAY -28: INJECTION
-28
-21
-14
-7
0
7
14
DAY POST-FIRST DOSE
DAY -30: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 1B

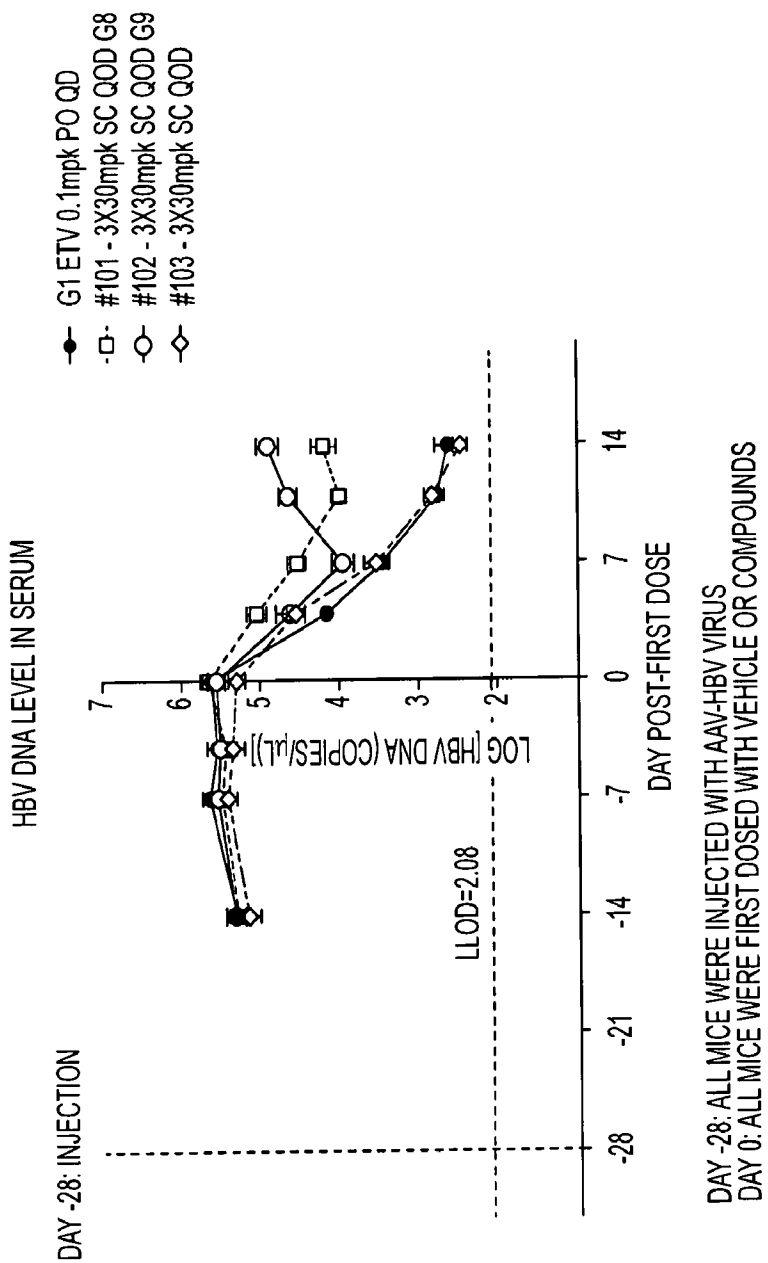

HBV DNA LEVEL IN SERUM
G1 ETV 0.1mpk PO QD
#101 - 3X30mpk SC QOD G8
#102 - 3X30mpk SC QOD G9
#103 - 3X30mpk SC QOD
LOG [HBV DNA (COPIES/μL)]
7
6
5
4
3
2
LLOD=2.08
DAY -28: INJECTION
-28
-21
-14
-7
0
7
14
DAY POST-FIRST DOSE
DAY -28: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 1C

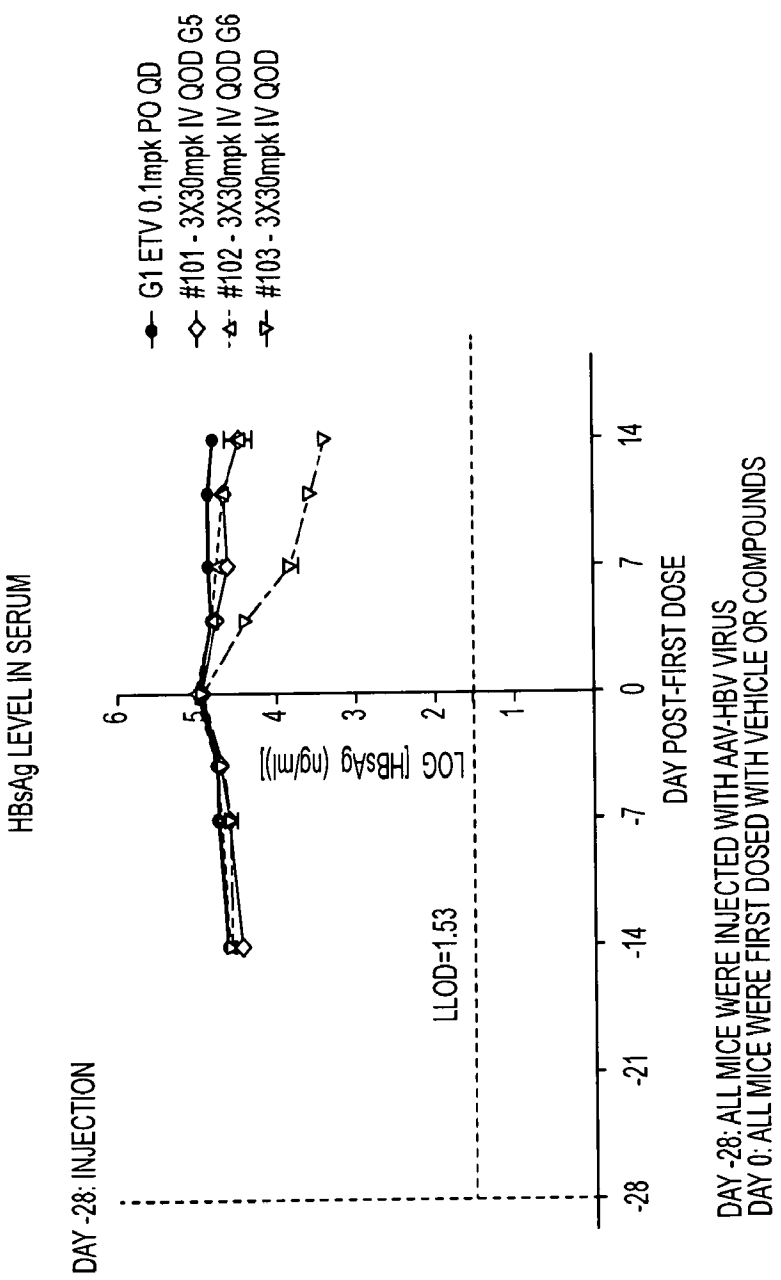

HBsAg LEVEL IN SERUM
G1 ETV 0.1mpk PO QD
#101 - 3X30mpk IV QOD G5
#102 - 3X30mpk IV QOD G6
#103 - 3X30mpk IV QOD
LOG [HBsAg (ng/ml)]
6
5
4
3
2
1
LLOD=1.53
DAY -28: INJECTION
-28
-21
-14
-7
0
7
14
DAY POST-FIRST DOSE
DAY -28: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 2A

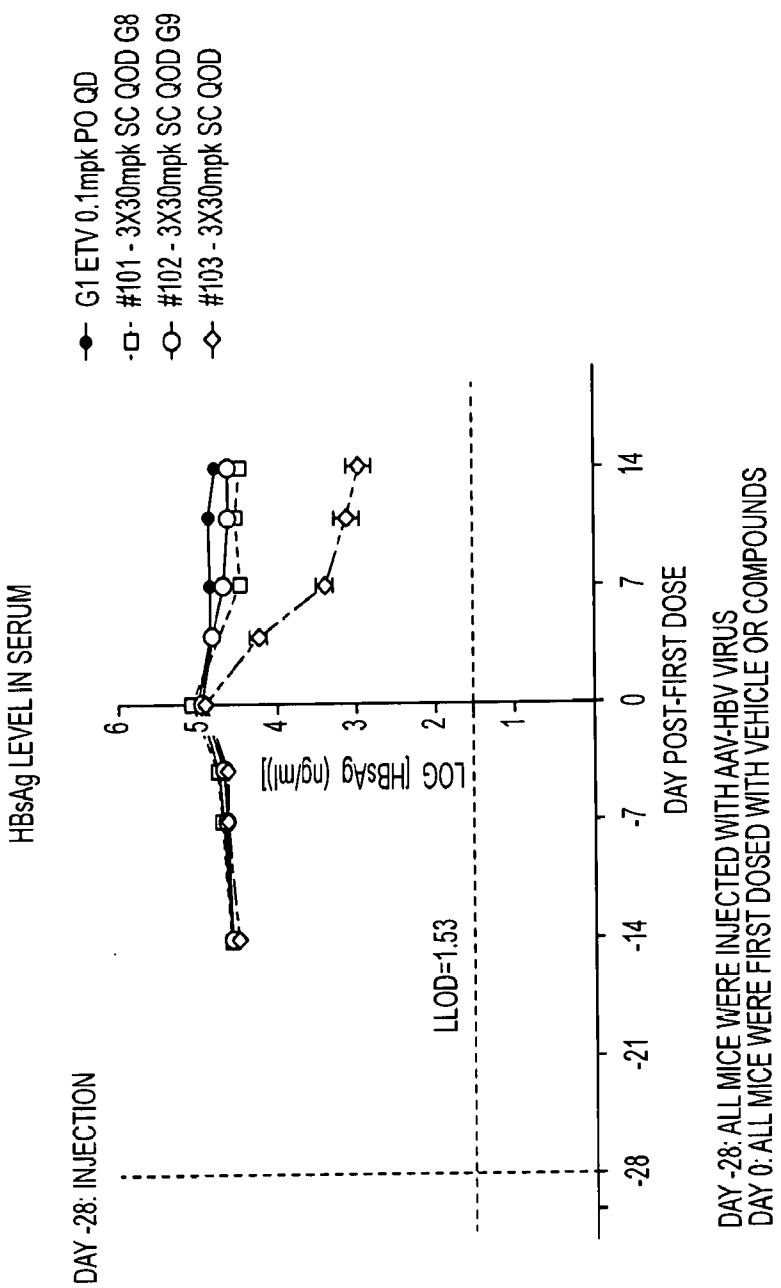
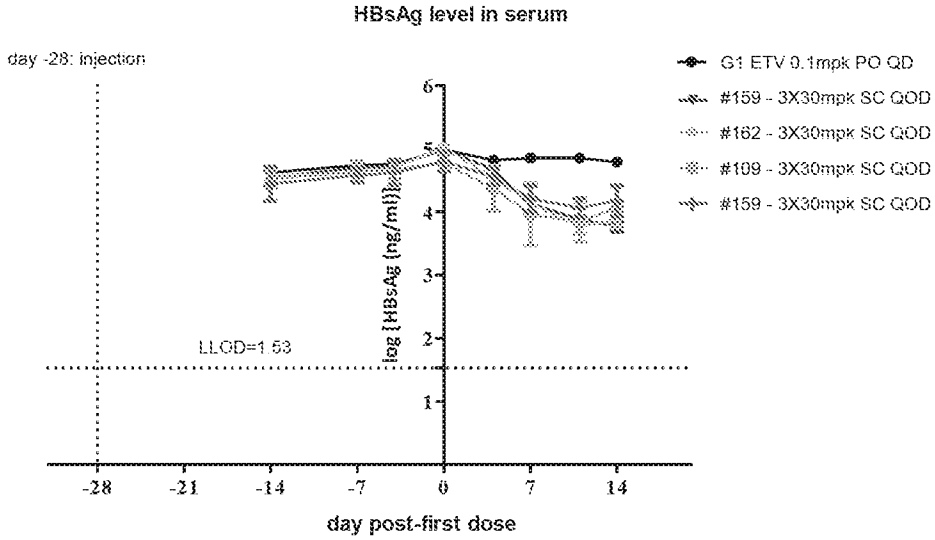

HBsAg LEVEL IN SERUM
G1 ETV 0.1mpk PO QD
#101 - 3X30mpk SC QOD G8
#102 - 3X30mpk SC QOD G9
#103 - 3X30mpk SC QOD
LOG [HBsAg (ng/ml)]
6
5
4
3
2
1
LLOD=1.53
DAY -28: INJECTION
-28
-21
-14
-7
0
7
14
DAY POST-FIRST DOSE
DAY -28: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 2B

FIG. 3



day -28: all mice were injected with AAV-HBV virus
day 0: all mice were first dosed with vehicle or compounds

	5'-mGpsmApsmUpsmUpsmApsmGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmApsmApsmApsmG-GalNAc-3'
	5'-mGpsmApsmUpsmUpsmApsGpsGpsCpsApsGpsApsGpsGpsTpsmGpsmApsmApsmApsmApsmApsmG-GalNAc-3'
	5'mApsmApsmGpsmApsmGpsApsGpsGpsTpsGps5meCpsGps5meCps5meCps5meCps5memCpsmGpsmUpsmGpsmG –GalNac 3'
	5'5memCpsmUpsmApsmGmGpsApsGpsTpsTps5meCps5meCpsGps5meCpsApsGpsmUpsmApsmUpsmGpsmG–GalNac 3'

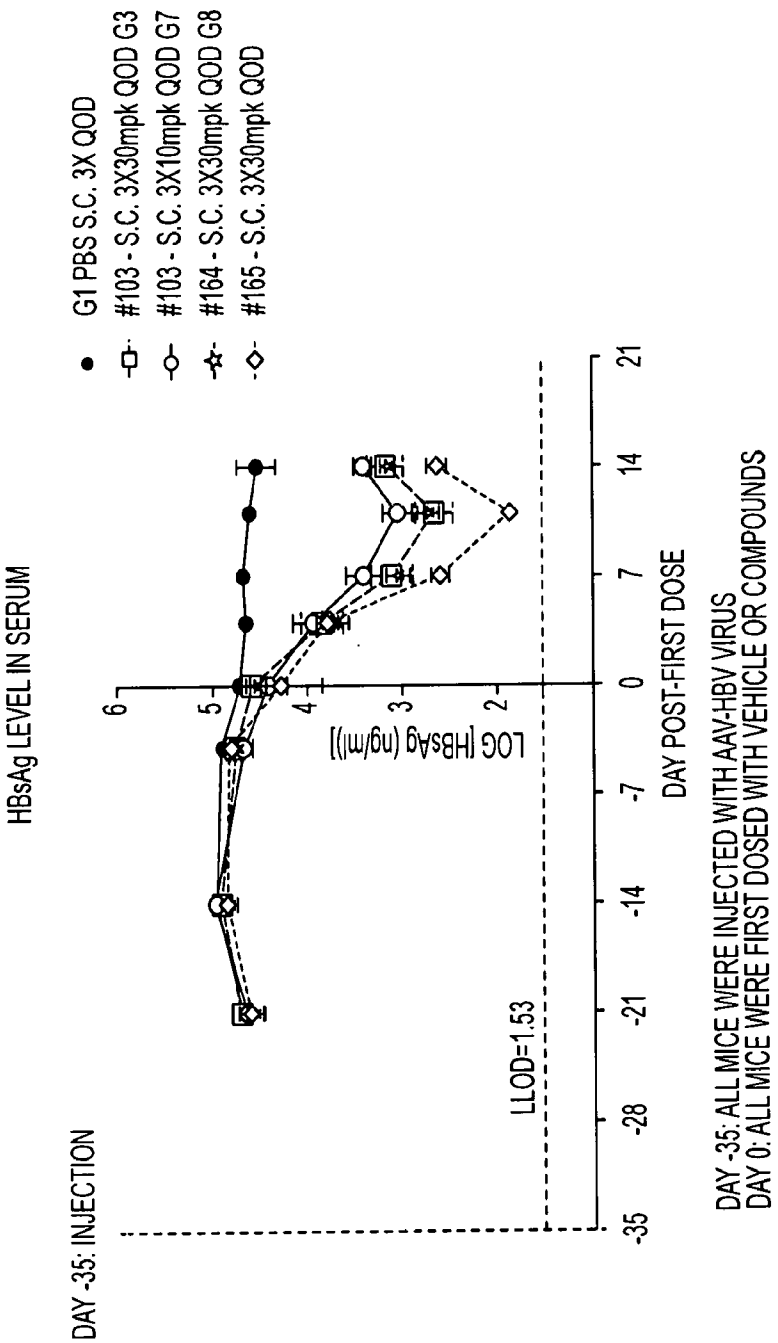

HBsAg LEVEL IN SERUM
G1 PBS S.C. 3X QOD
#103 - S.C. 3X30mpk QOD G3
#103 - S.C. 3X10mpk QOD G7
#164 - S.C. 3X30mpk QOD G8
#165 - S.C. 3X30mpk QOD
LOG [HBsAg (ng/ml)]
6
5
4
3
2
LLOD=1.53
DAY -35: INJECTION
-35
-28
-21
-14
-7
0
7
14
21
DAY POST-FIRST DOSE
DAY -35: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 4A

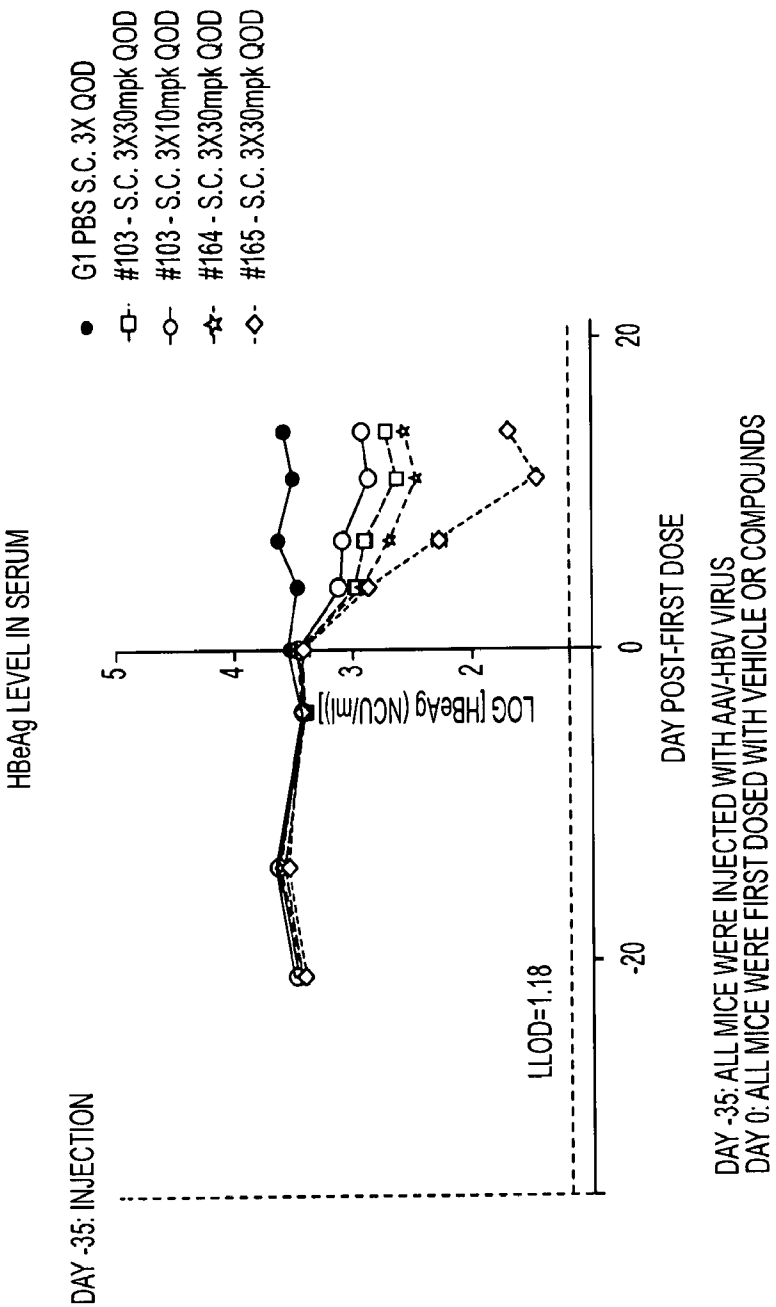

HBeAg LEVEL IN SERUM
G1 PBS S.C. 3X QOD
#103 - S.C. 3X30mpk QOD
#103 - S.C. 3X10mpk QOD
#164 - S.C. 3X30mpk QOD
#165 - S.C. 3X30mpk QOD
LOG [HBeAg (NCU/ml)]
5
4
3
2
LLOD=1.18
DAY -35: INJECTION
-20
0
20
DAY POST-FIRST DOSE
DAY -35: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 4B

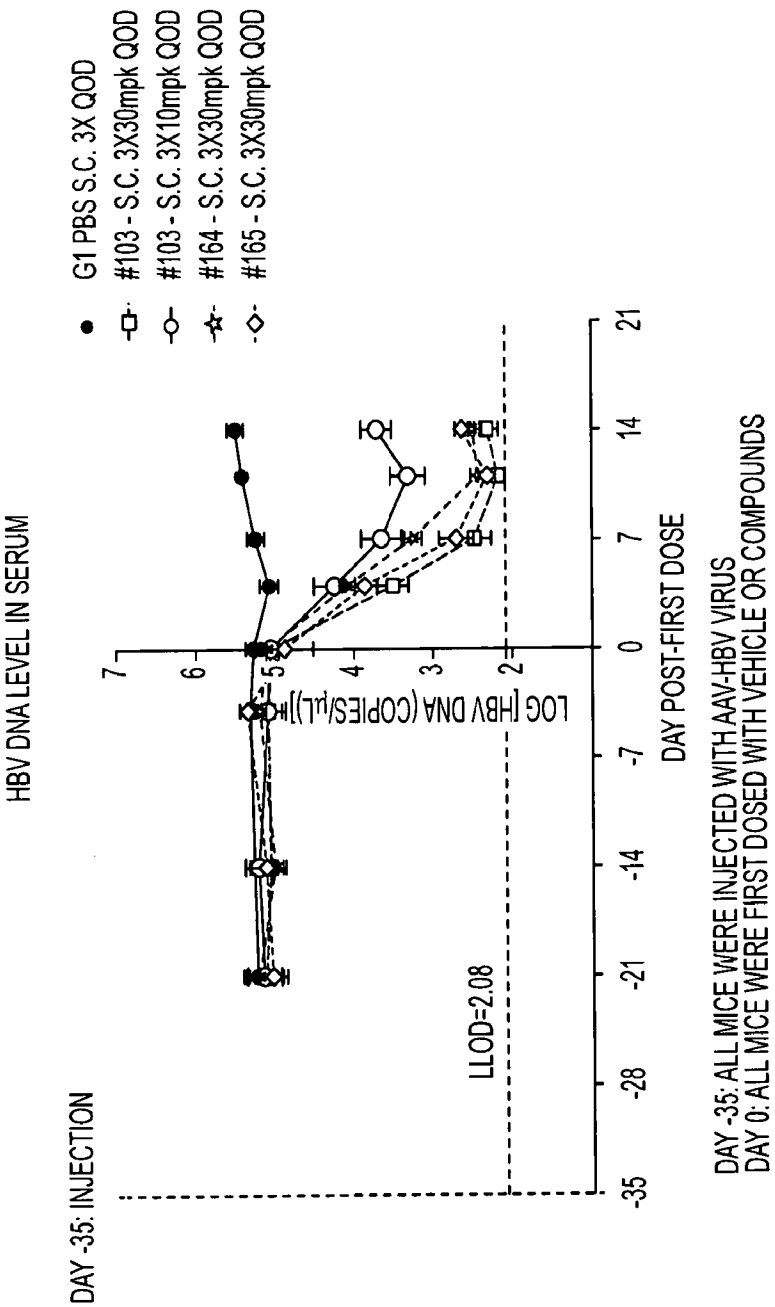

HBV DNA LEVEL IN SERUM
G1 PBS S.C. 3X QOD
#103 - S.C. 3X30mpk QOD
#103 - S.C. 3X10mpk QOD
#164 - S.C. 3X30mpk QOD
#165 - S.C. 3X30mpk QOD
LOG [HBV DNA (COPIES/μL)]
7
6
5
4
3
2
LLOD=2.08
DAY -35: INJECTION
-35
-28
-21
-14
-7
0
7
14
21
DAY POST-FIRST DOSE
DAY -35: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 4C

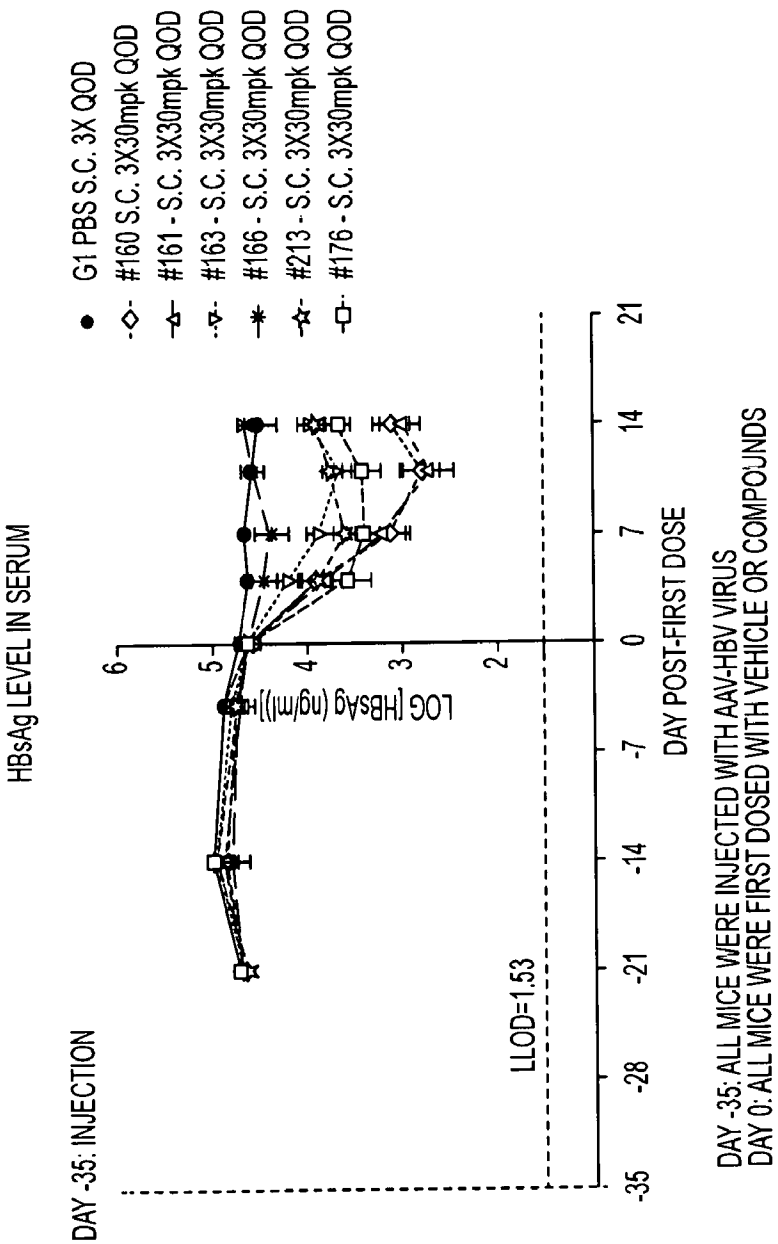

HBsAg LEVEL IN SERUM
G1 PBS S.C. 3X QOD
#160 S.C. 3X30mpk QOD
#161 - S.C. 3X30mpk QOD
#163 - S.C. 3X30mpk QOD
#166 - S.C. 3X30mpk QOD
#213 - S.C. 3X30mpk QOD
#176 - S.C. 3X30mpk QOD
LOG [HBsAg (ng/ml)]
6
5
4
3
2
DAY -35: INJECTION
LLOD=1.53
-35
-28
-21
-14
-7
0
7
14
21
DAY POST-FIRST DOSE
DAY -35: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 5A

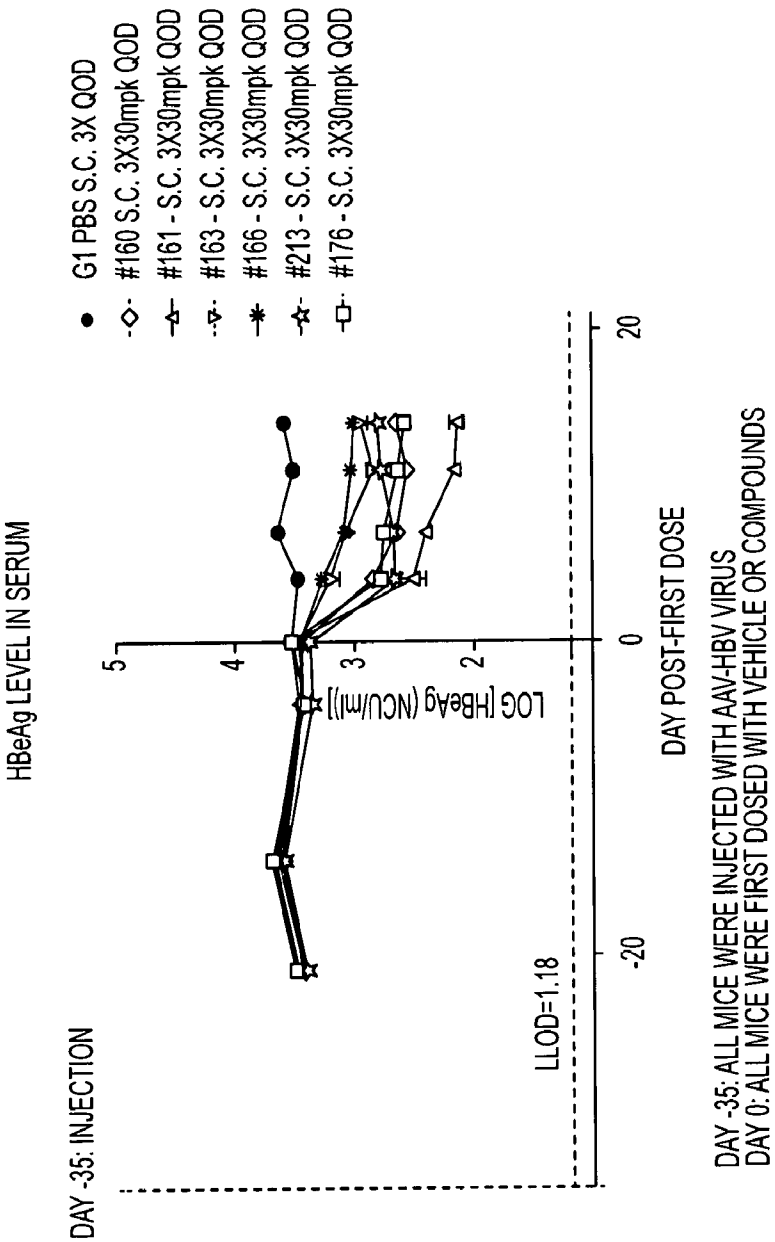

HBeAg LEVEL IN SERUM
G1 PBS S.C. 3X QOD
#160 S.C. 3X30mpk QOD
#161 - S.C. 3X30mpk QOD
#163 - S.C. 3X30mpk QOD
#166 - S.C. 3X30mpk QOD
#213 - S.C. 3X30mpk QOD
#176 - S.C. 3X30mpk QOD
LOG [HBeAg (NCU/ml)]
5
4
3
2
LLOD=1.18
DAY -35: INJECTION
-20
0
20
DAY POST-FIRST DOSE
DAY -35: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 5B

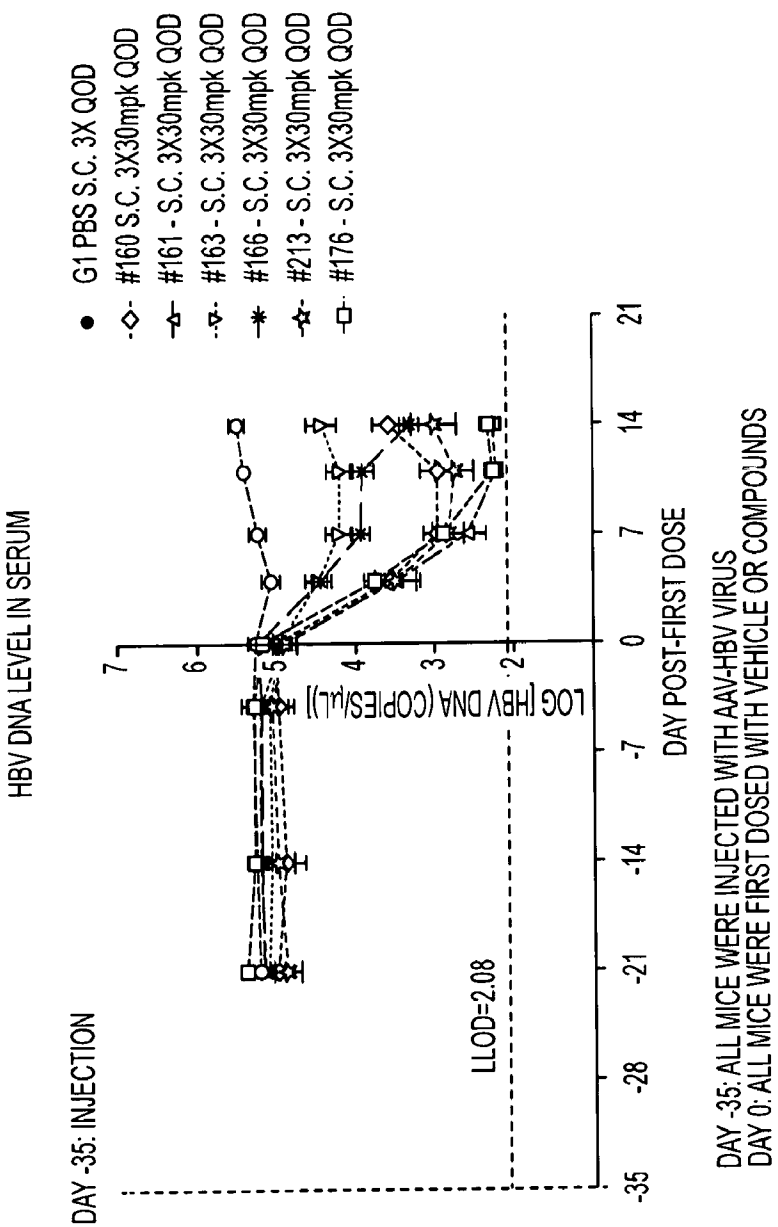

HBV DNA LEVEL IN SERUM
G1 PBS S.C. 3X QOD
#160 S.C. 3X30mpk QOD
#161 - S.C. 3X30mpk QOD
#163 - S.C. 3X30mpk QOD
#166 - S.C. 3X30mpk QOD
#213 - S.C. 3X30mpk QOD
#176 - S.C. 3X30mpk QOD
LOG [HBV DNA (COPIES/μL)]
7
6
5
4
3
2
LLOD=2.08
DAY -35: INJECTION
-35
-28
-21
-14
-7
0
7
14
21
DAY POST-FIRST DOSE
DAY -35: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 5C

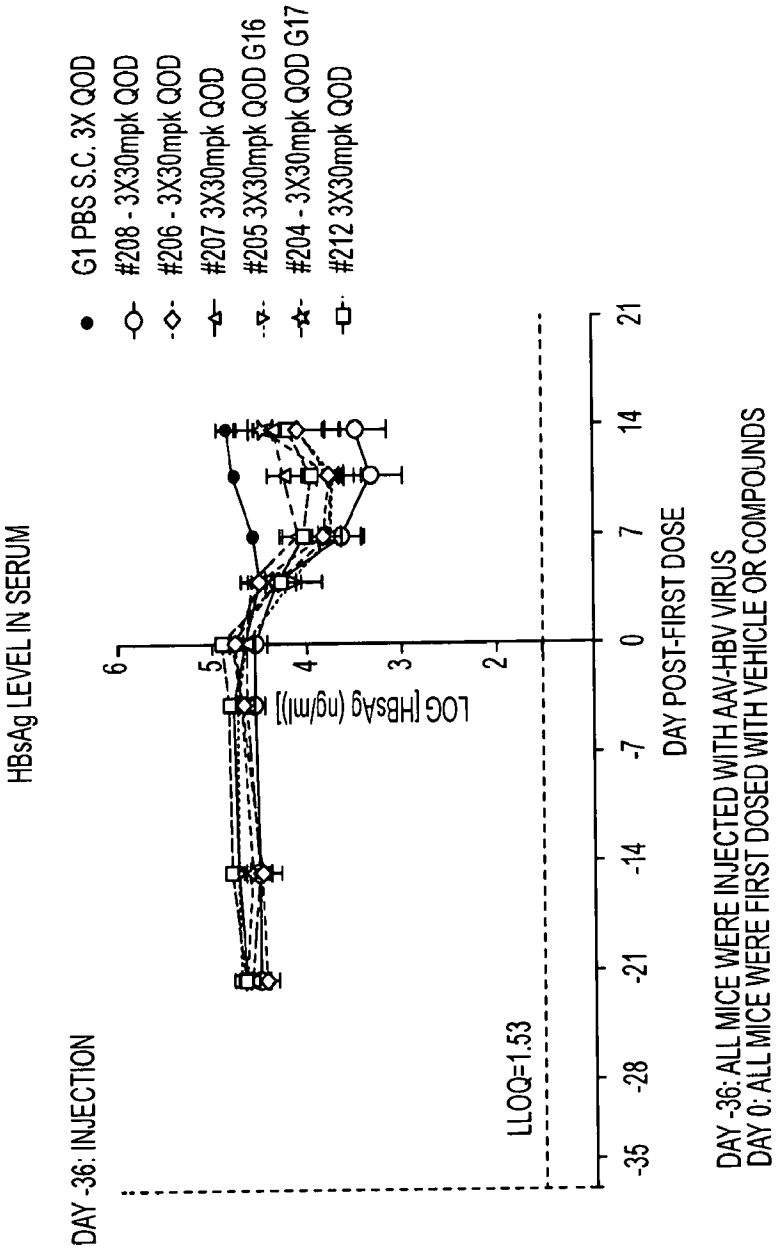

HBsAg LEVEL IN SERUM
G1 PBS S.C. 3X QOD
#208 - 3X30mpk QOD
#206 - 3X30mpk QOD
#207 3X30mpk QOD
#205 3X30mpk QOD G16
#204 - 3X30mpk QOD G17
#212 3X30mpk QOD
LOG [HBsAg (ng/ml)]
6
5
4
3
2
DAY -36: INJECTION
LLOQ=1.53
-35
-28
-21
-14
-7
0
7
14
21
DAY POST-FIRST DOSE
DAY -36: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 6A

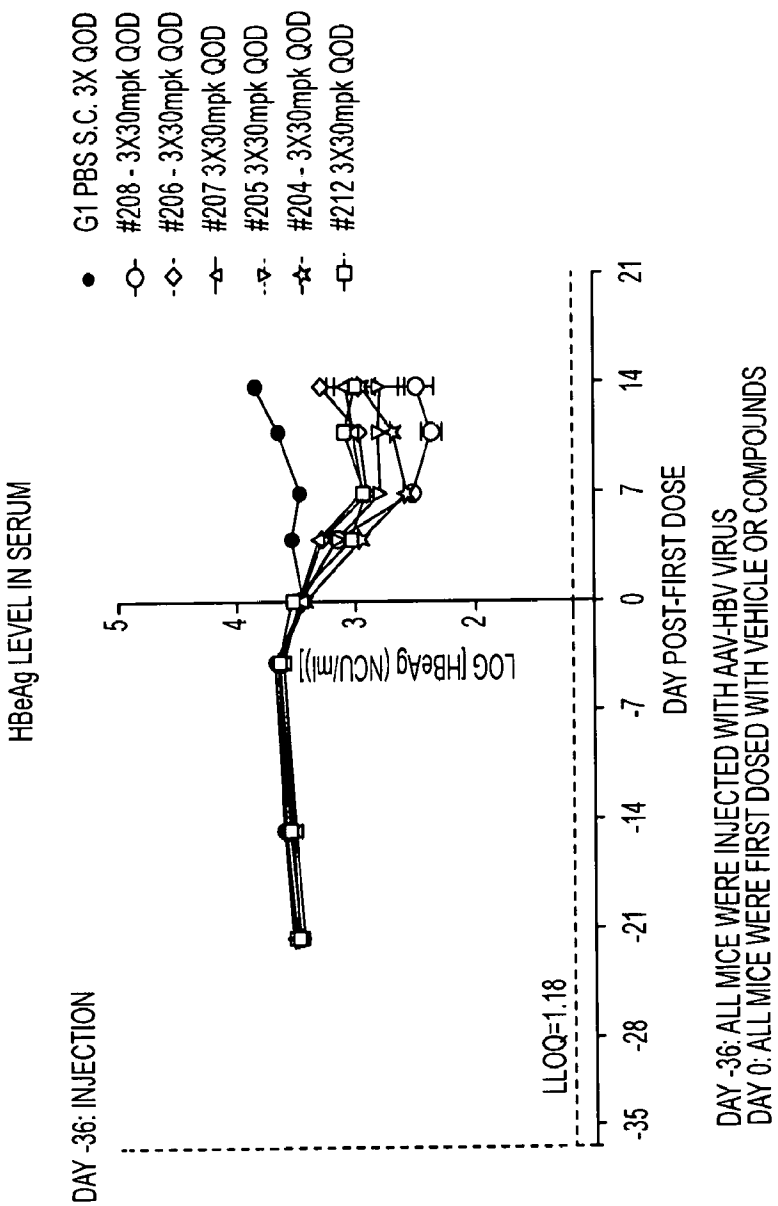

HBeAg LEVEL IN SERUM
G1 PBS S.C. 3X QOD
#208 - 3X30mpk QOD
#206 - 3X30mpk QOD
#207 3X30mpk QOD
#205 3X30mpk QOD
#204 - 3X30mpk QOD
#212 3X30mpk QOD
LOG [HBeAg (NCU/ml)]
5
4
3
2
DAY -36: INJECTION
LLOQ=1.18
-35
-28
-21
-14
-7
0
7
14
21
DAY POST-FIRST DOSE
DAY -36: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 6B

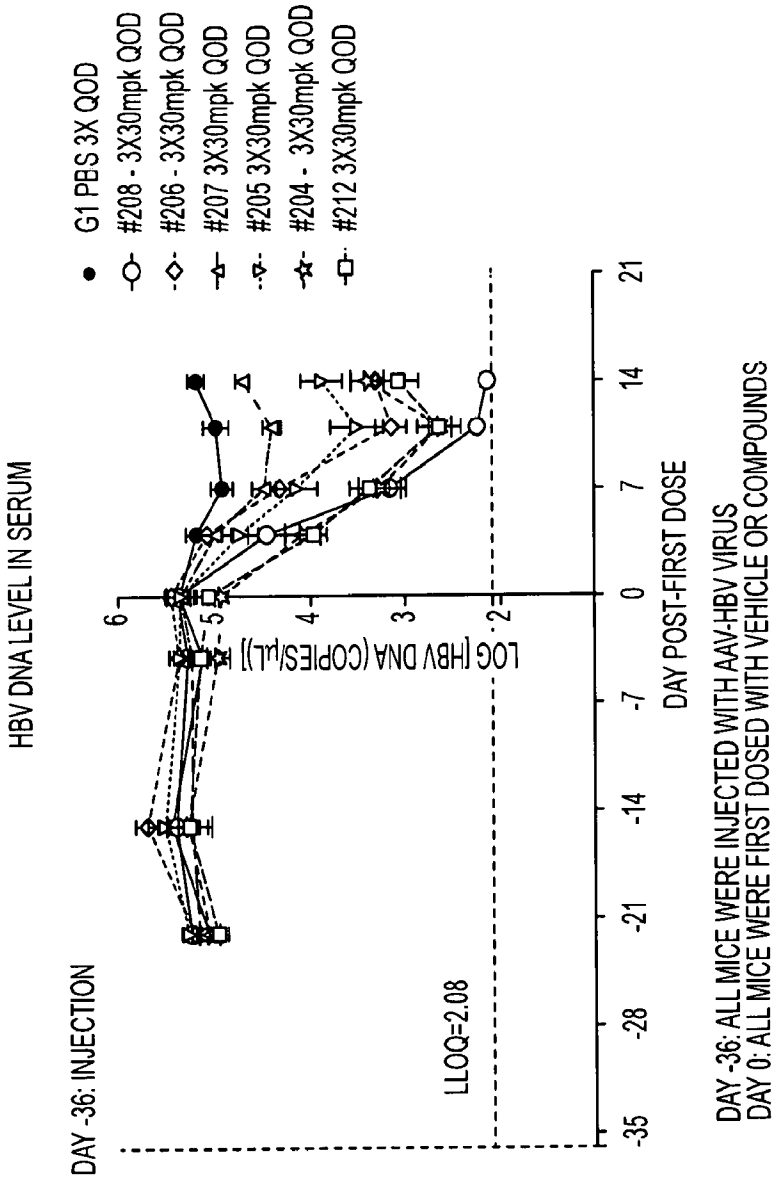

HBV DNA LEVEL IN SERUM
G1 PBS 3X QOD
#208 - 3X30mpk QOD
#206 - 3X30mpk QOD
#207 3X30mpk QOD
#205 3X30mpk QOD
#204 - 3X30mpk QOD
#212 3X30mpk QOD
LOG [HBV DNA (COPIES/μL)]
6
5
4
3
2
DAY -36: INJECTION
LLOQ=2.08
-35
-28
-21
-14
-7
0
7
14
21
DAY POST-FIRST DOSE
DAY -36: ALL MICE WERE INJECTED WITH AAV-HBV VIRUS
DAY 0: ALL MICE WERE FIRST DOSED WITH VEHICLE OR COMPOUNDS

FIG. 6C

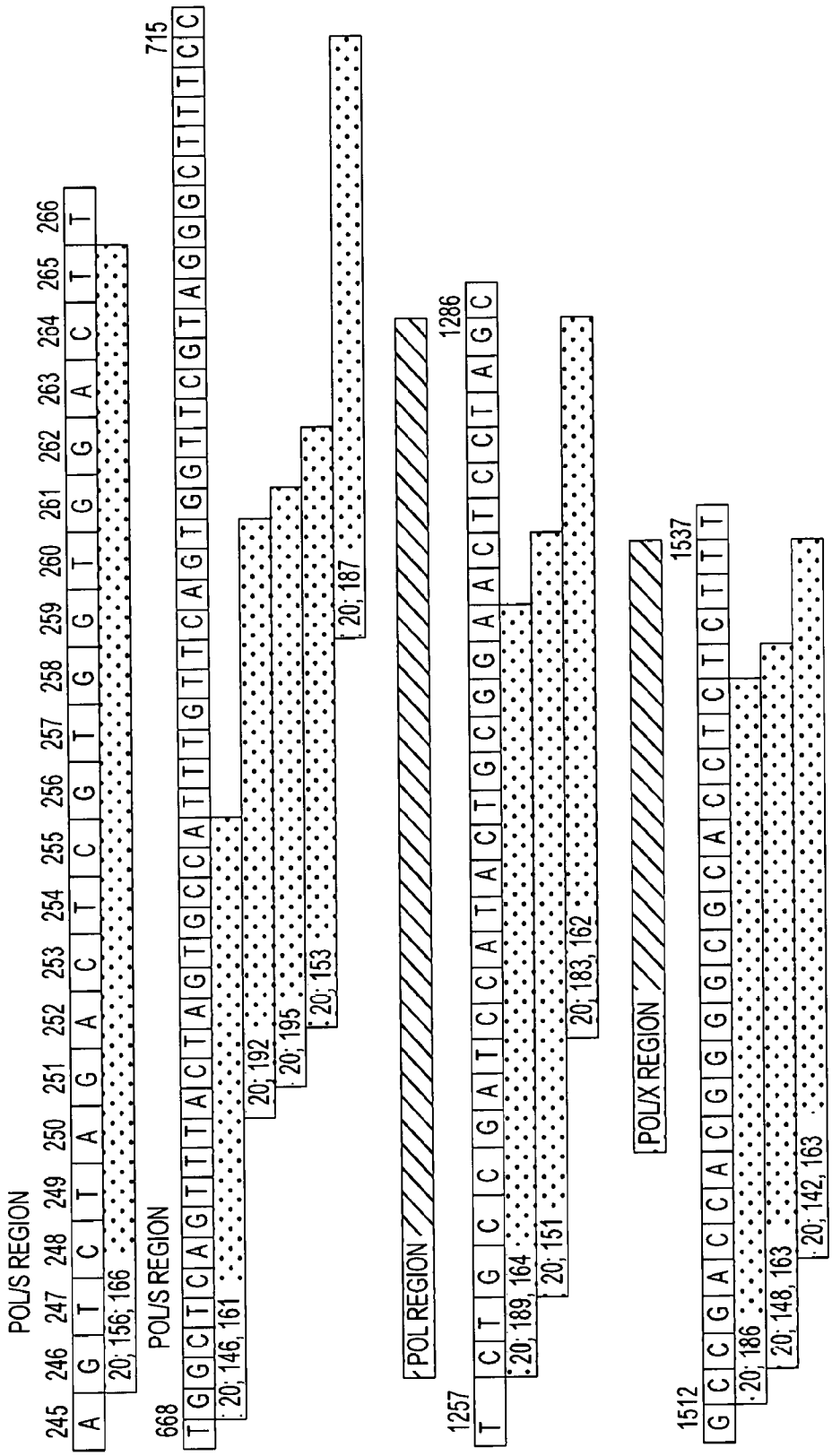
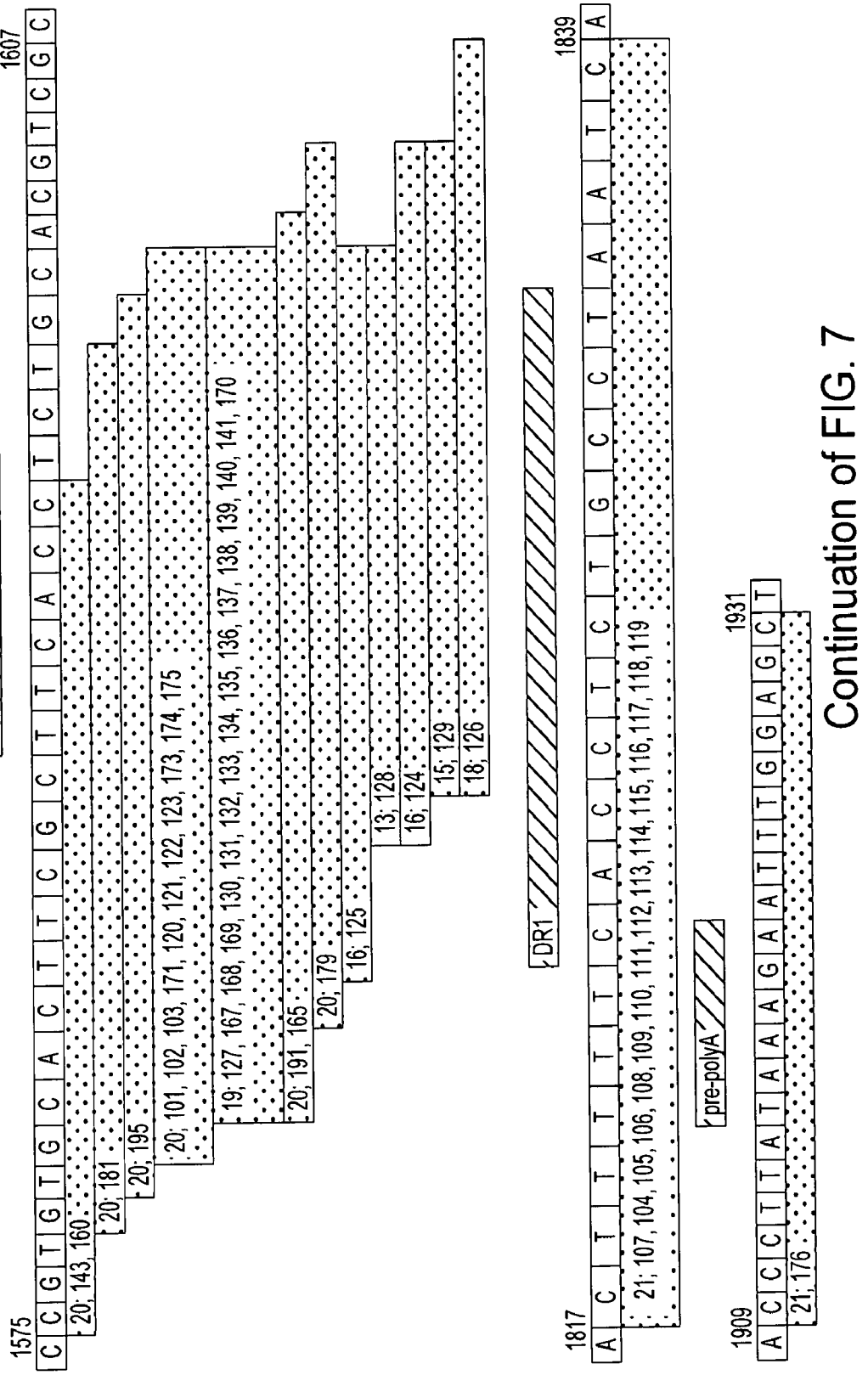

POL/S REGION
245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266
A G T C T A G A C T C G T G T G G G A C T T
20; 156; 166
POL/S REGION
668 715
T G G C T C A G T T T A C T A G T G C C A T T T G T T C A G T A G G G C T T T C C
20; 146, 161
20; 192
20; 195
20; 153
20; 187
POL REGION
1257 1286
T C T G C C G A T A C T C C A T A C T G C G G A A A C T C C T A G C
20; 189, 164
20; 151
20; 183, 162
POL/X REGION
1512 1537
G C C G A C A C G G G G C G C A C C T C T T T
20; 186
20; 148, 163
20; 142, 163

FIG. 7



Continuation of FIG. 7

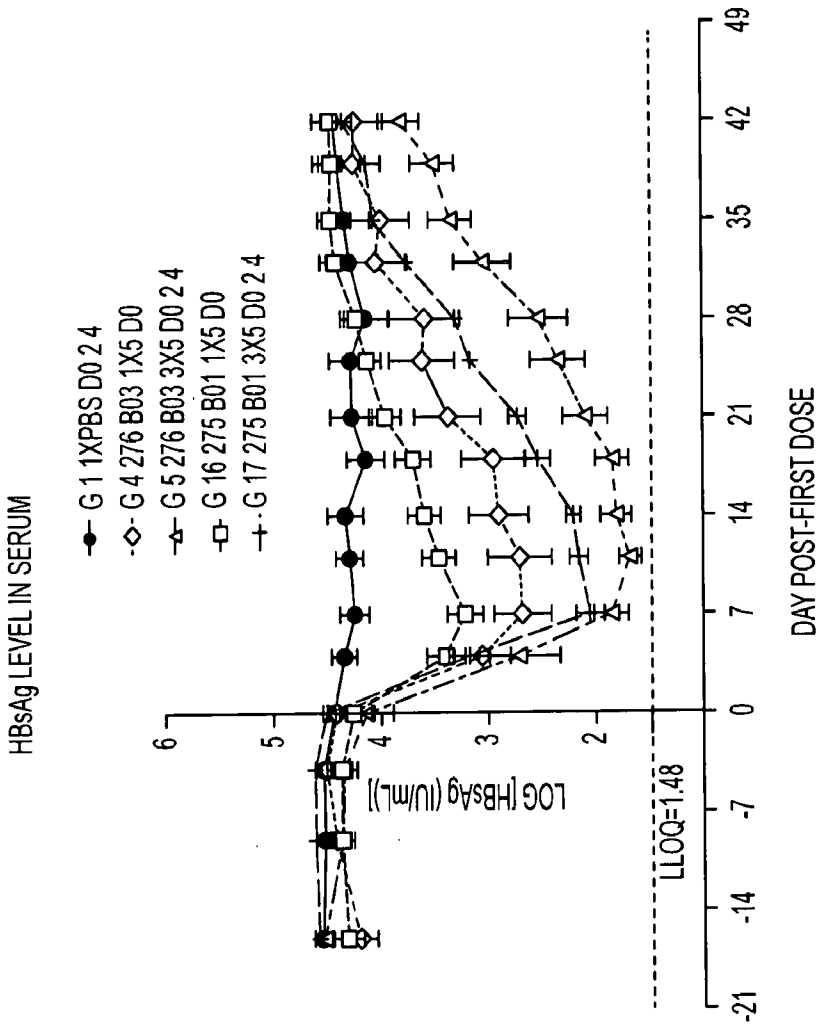

HBsAg LEVEL IN SERUM
G 1 1XPBS D0 2 4
G 4 276 B03 1X5 D0
G 5 276 B03 3X5 D0 2 4
G 16 275 B01 1X5 D0
G 17 275 B01 3X5 D0 2 4
LOG [HBsAg (IU/mL)]
6
5
4
3
2
LLOQ=1.48
-21
-14
-7
0
7
14
21
28
35
42
49
DAY POST-FIRST DOSE

FIG. 8

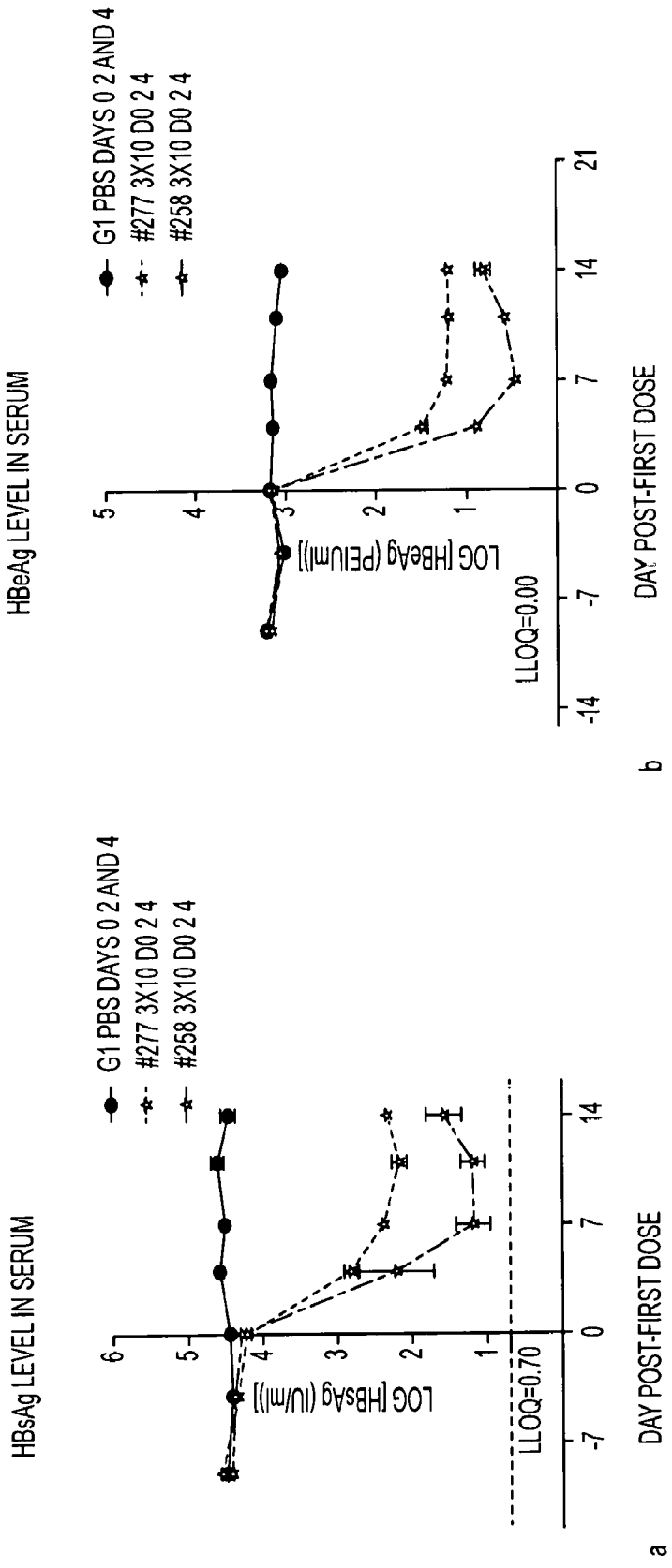

HBeAg LEVEL IN SERUM
G1 PBS DAYS 0 2 AND 4
#277 3X10 D0 2 4
#258 3X10 D0 2 4
LOG [HBeAg (PEIU/ml)]
LLOQ=0.00
DAY POST-FIRST DOSE
b
HBsAg LEVEL IN SERUM
G1 PBS DAYS 0 2 AND 4
#277 3X10 D0 2 4
#258 3X10 D0 2 4
LOG [HBsAg (IU/ml)]
LLOQ=0.70
DAY POST-FIRST DOSE
a

FIG. 9B

FIG. 9A

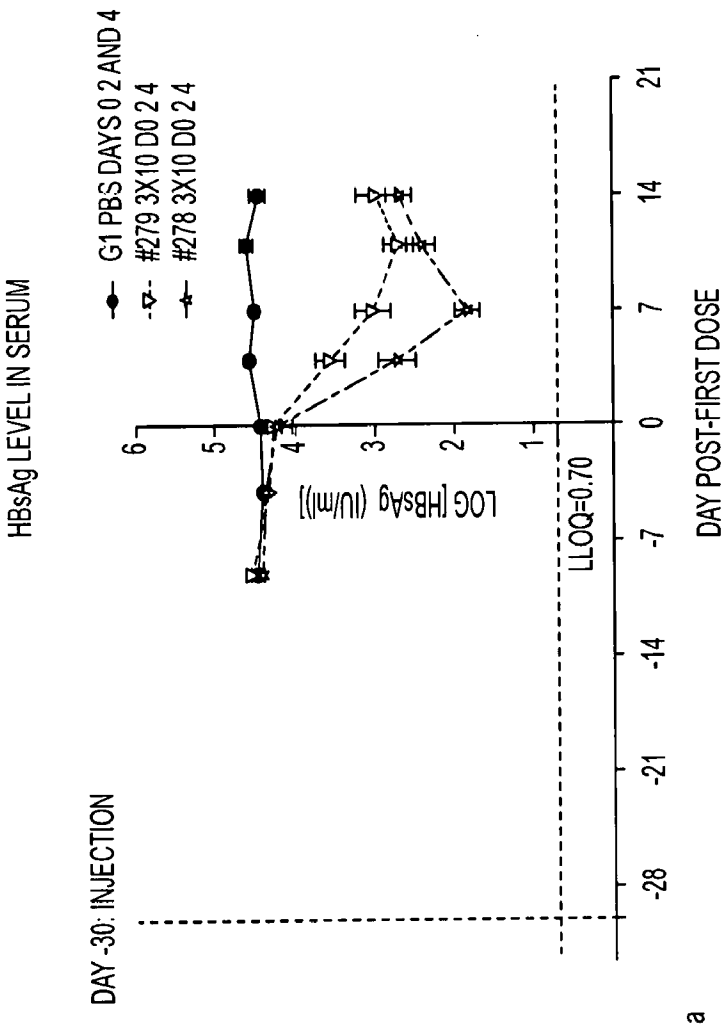

HBsAg LEVEL IN SERUM
G1 PBS DAYS 0 2 AND 4
#279 3X10 D0 2 4
#278 3X10 D0 2 4
LOG [HBsAg (IU/ml)]
6
5
4
3
2
1
LLOQ=0.70
DAY -30: INJECTION
-28
-21
-14
-7
0
7
14
21
DAY POST-FIRST DOSE
a

FIG. 10A

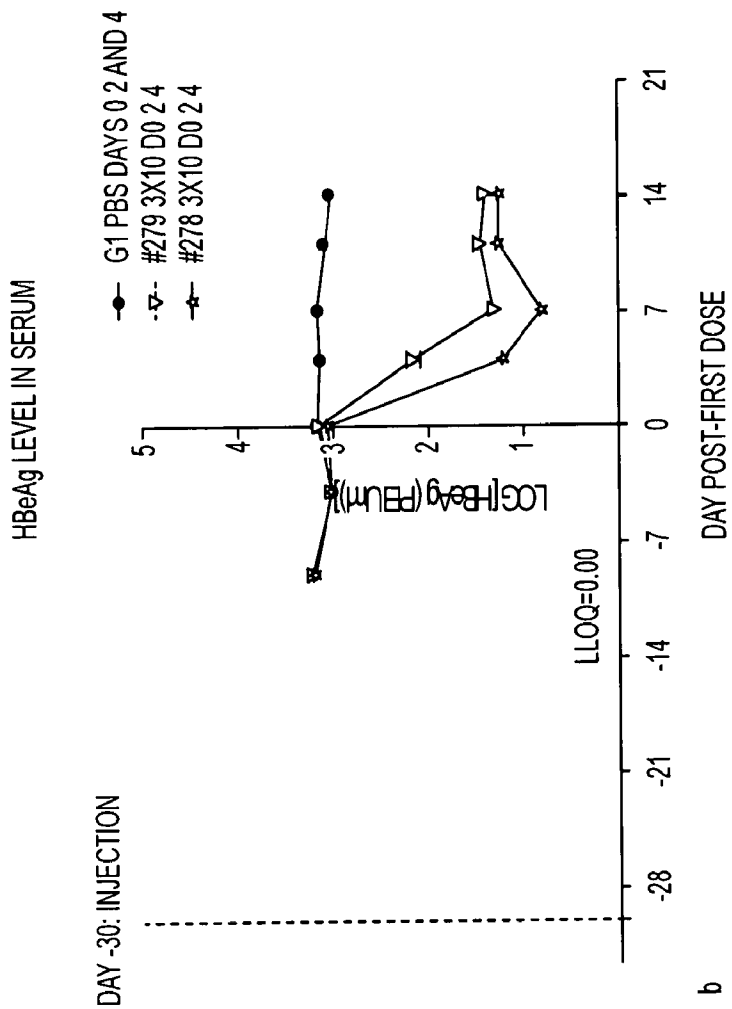

HBeAg LEVEL IN SERUM
G1 PBS DAYS 0 2 AND 4
#279 3X10 D0 2 4
#278 3X10 D0 2 4
LOG[HBeAg (PEUnit)]
5
4
3
2
1
LLOQ=0.00
DAY -30: INJECTION
-28
-21
-14
-7
0
7
14
21
DAY POST-FIRST DOSE
b

FIG. 10B

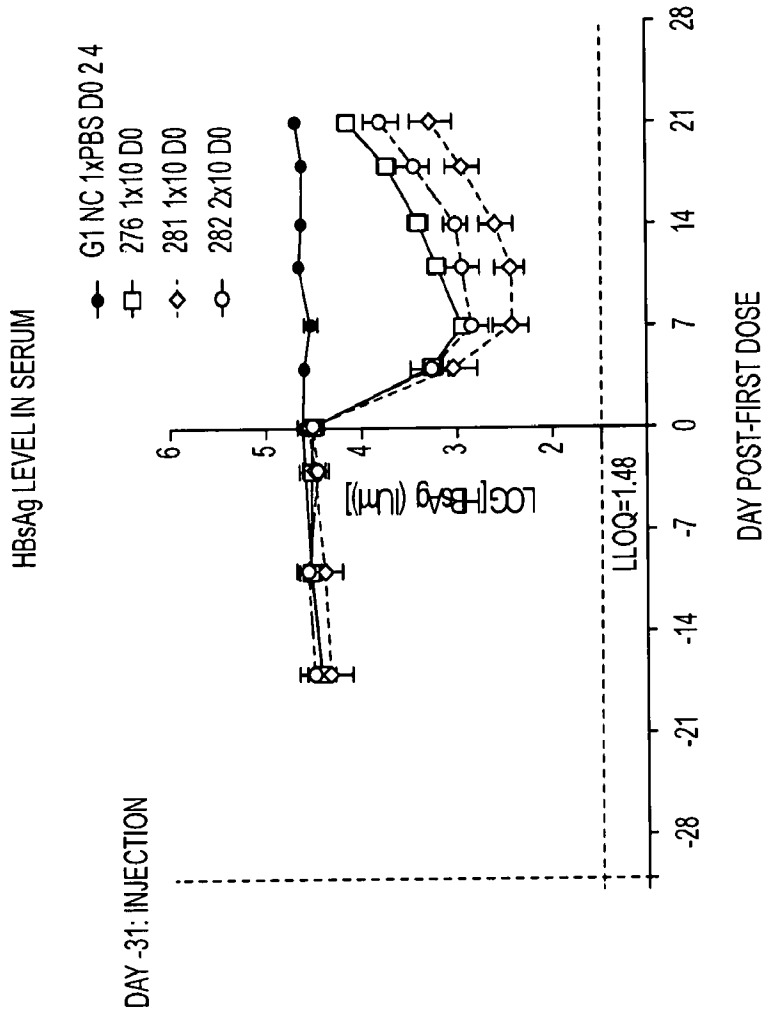

HBsAg LEVEL IN SERUM
G1 NC 1xPBS D0 2 4
276 1x10 D0
281 1x10 D0
282 2x10 D0
Log[HBsAg (IU/ml)]
6
5
4
3
2
LLOQ=1.48
DAY -31: INJECTION
-28
-21
-14
-7
0
7
14
21
28
DAY POST-FIRST DOSE

FIG. 11A

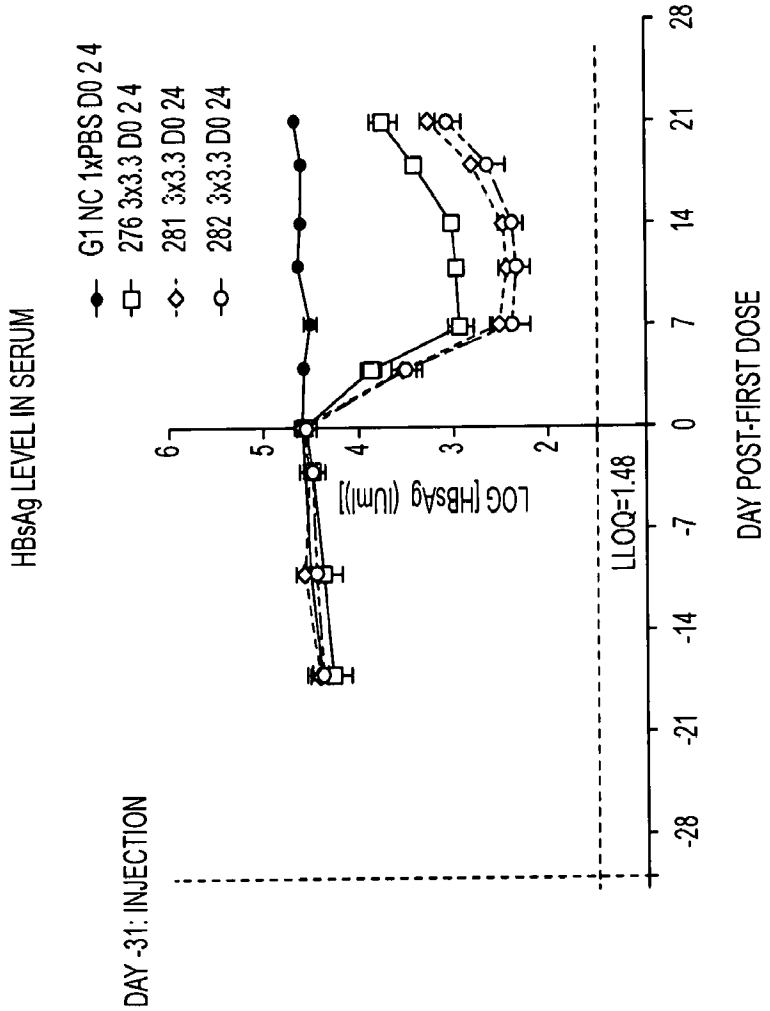

HBsAg LEVEL IN SERUM
G1 NC 1xPBS D0 2 4
276 3x3.3 D0 2 4
281 3x3.3 D0 24
282 3x3.3 D0 24
LOG [HBsAg (IU/ml)]
6
5
4
3
2
LLOQ=1.48
DAY -31: INJECTION
-28
-21
-14
-7
0
7
14
21
28
DAY POST-FIRST DOSE

FIG. 11B

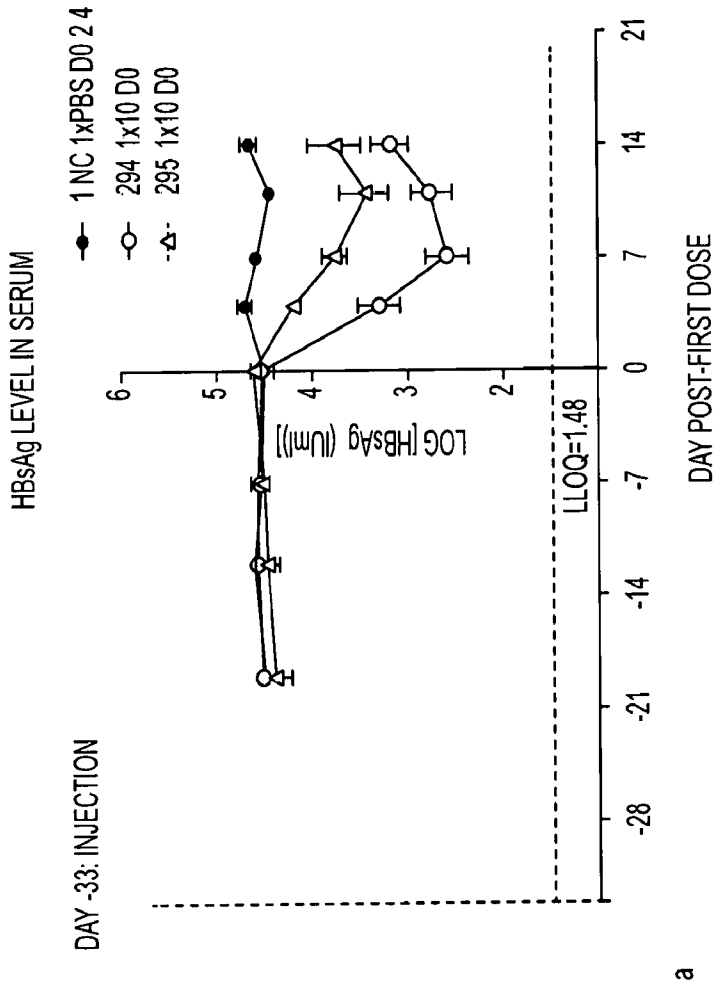

HBsAg LEVEL IN SERUM
1 NC 1xPBS D0 2 4
294 1x10 D0
295 1x10 D0
LOG [HBsAg (IU/ml)]
6
5
4
3
2
LLOQ=1.48
DAY -33: INJECTION
-28
-21
-14
-7
0
7
14
21
DAY POST-FIRST DOSE
a

FIG. 12A

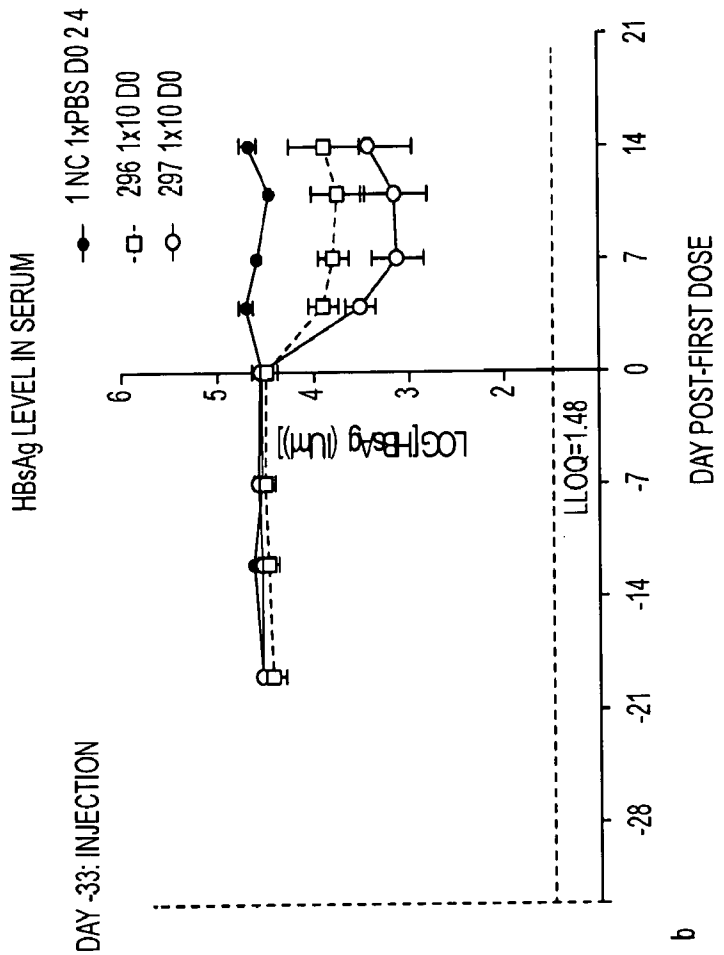

HBsAg LEVEL IN SERUM
1 NC 1xPBS D0 2 4
296 1x10 D0
297 1x10 D0
LOG[HBsAg (IU/ml)]
6
5
4
3
2
LLOQ=1.48
DAY -33: INJECTION
-28
-21
-14
-7
0
7
14
21
DAY POST-FIRST DOSE
b

FIG. 12B

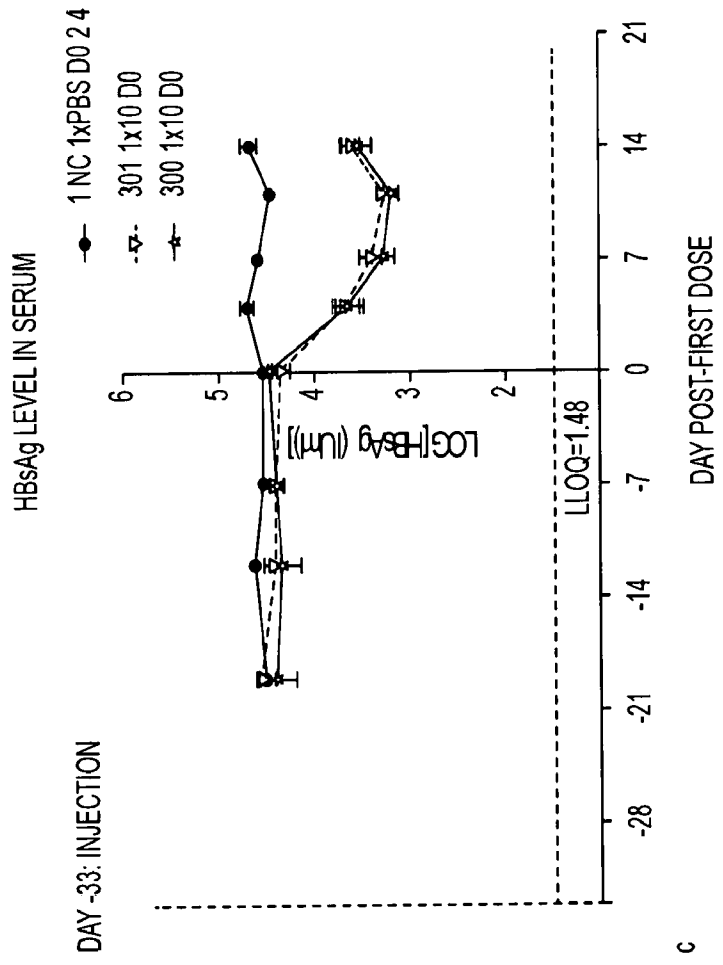

HBsAg LEVEL IN SERUM
1 NC 1xPBS D0 2 4
301 1x10 D0
300 1x10 D0
LOG[HBsAg (IU/ml)]
6
5
4
3
2
LLOQ=1.48
-28
-21
-14
-7
0
7
14
21
DAY POST-FIRST DOSE
DAY -33: INJECTION
C

FIG. 12C