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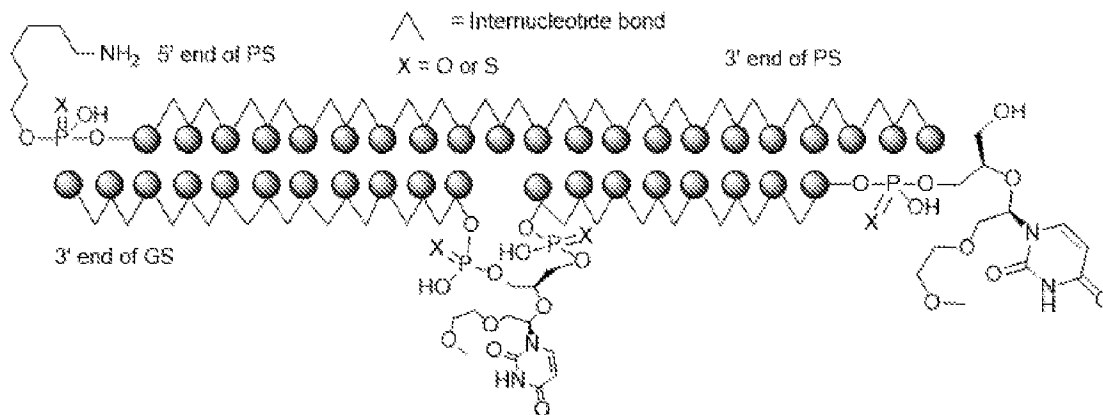
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(54) Titre : AMIDITES D'UNA ET LEURS UTILISATIONS
(54) Title: UNA AMIDITES AND USES THEREOF

FIG. 1B



(57) **Abrégé/Abstract:**

Disclosed herein are compositions and pharmaceutical formulations that comprise a binding moiety conjugated to a modified polynucleic acid molecule and a polymer. Also described herein include methods for treating a disease which utilize a composition or a pharmaceutical formulation comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer.



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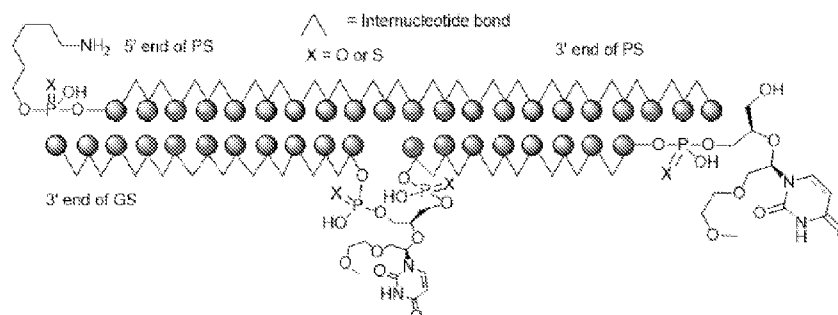
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
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(54) Title: UNA AMIDITES AND USES THEREOF

FIG. 1B



(57) Abstract: Disclosed herein are compositions and pharmaceutical formulations that comprise a binding moiety conjugated to a modified polynucleic acid molecule and a polymer. Also described herein include methods for treating a disease which utilize a composition or a pharmaceutical formulation comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer.



WO 2020/247818 A1

UNA AMIDITES AND USES THEREOF

CROSS-REFERENCE

[0001] This application claims benefit of U.S. Provisional Patent Application No. 62/858,289 filed June 6, 2019, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

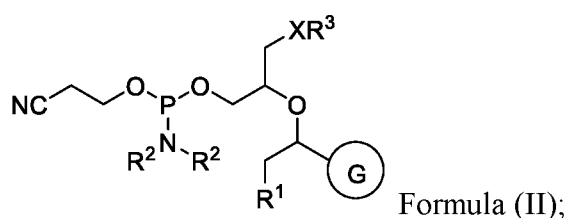
[0001.1] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 5, 2020, is named 45532-735_601_SL.txt and is 3,172,165 bytes in size.

BACKGROUND OF THE DISCLOSURE

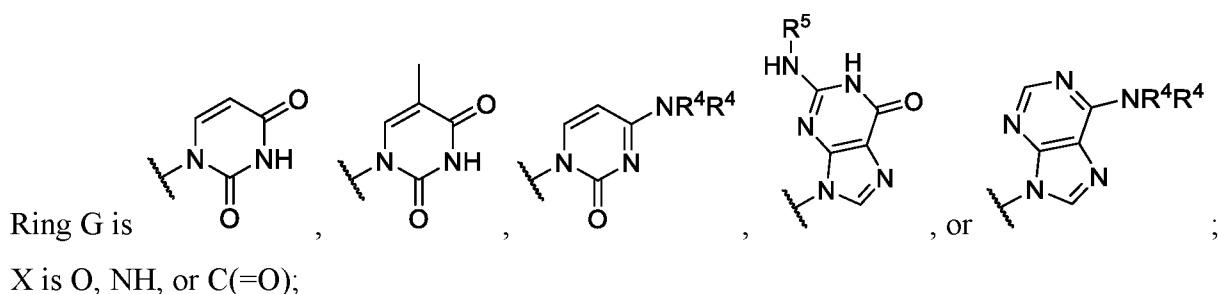
[0002] Gene suppression by RNA-induced gene silencing provides several levels of control: transcription inactivation, small interfering RNA (siRNA)-induced mRNA degradation, and siRNA-induced transcriptional attenuation. In some instances, RNA interference (RNAi) provides long lasting effect over multiple cell divisions. As such, RNAi represents a viable method useful for drug target validation, gene function analysis, pathway analysis, and disease therapeutics.

SUMMARY OF THE DISCLOSURE

[0003] Disclosed herein, in certain embodiments, is a compound according to Formula (II):



wherein,



R¹ is hydrogen, deuterium, halogen, substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, substituted or unsubstituted C₁-C₁₀ fluoroalkyl, substituted or unsubstituted C₁-C₂₀ heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl,

substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted -O-aryl, -OR⁶, -C(=O)R⁶, -C(=O)OR⁶, -C(=O)NR⁷R⁷, -OCH₂C(=O)R⁶, -OCH₂C(=O)OR⁶, or -OCH₂C(=O)NR⁷R⁷;

each R² is independently hydrogen, deuterium, or substituted or unsubstituted C₁-C₁₀ alkyl; or two R² are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C₃-C₁₀ heterocycloalkyl;

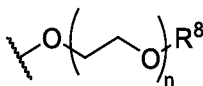
R³ is hydrogen, 4,4'-dimethoxytrityl (DMT), -R⁶, -OR⁶, or -NR⁷R⁷;

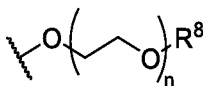
each R⁴ is independently hydrogen, substituted or unsubstituted C₁-C₆alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted benzoyl;

R⁵ is -C(=O)R⁶;

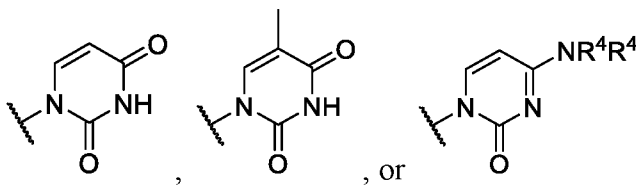
each R⁶ and R⁷ are independently hydrogen, substituted or unsubstituted C₁-C₆alkyl, substituted or unsubstituted C₁-C₆fluoroalkyl, substituted or unsubstituted C₃-C₆cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

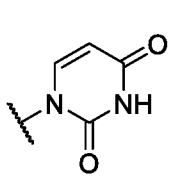
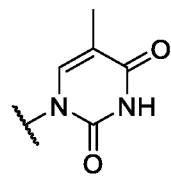
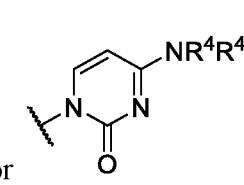
[0004] In some instances, R¹ is halogen, substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₁-C₁₀ fluoroalkyl, substituted or unsubstituted C₁-C₂₀ heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted -O-aryl, -OH, -OR⁶, -C(=O)R⁶, -C(=O)OR⁶, -C(=O)NR⁷R⁷, -OCH₂C(=O)R⁶, or -OCH₂C(=O)OR⁶, or -OCH₂C(=O)NR⁷R⁷. In some instances, R¹ is halogen, substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₁-C₂₀ heteroalkyl, substituted or unsubstituted -O-aryl, -OCH₂C(=O)R⁶, or -OCH₂C(=O)OR⁶, or -OCH₂C(=O)NR⁷R⁷. In some instances, R¹ is halogen or substituted or unsubstituted C₁-C₂₀ heteroalkyl.

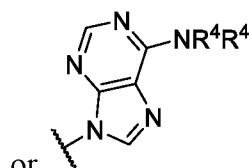
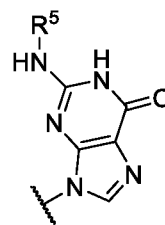
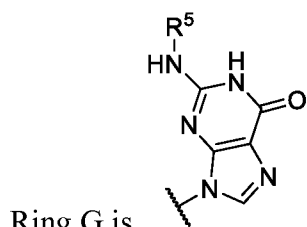


[0005] In some instances, R¹ is , where n is 0, 1, 2, 3, 4, or 5, and R⁸ is hydrogen or substituted or unsubstituted C₁-C₃ alkyl. In some embodiments, R⁸ is substituted or unsubstituted C₁-C₃ alkyl. In some embodiments, R⁸ is methyl or ethyl. In some embodiments, n is 1 or 2.

[0006] In some instances, R¹ is -OCH₂C(=O)NR⁷R⁷; wherein each R⁷ is independently hydrogen or substituted or unsubstituted C₁-C₃ alkyl.

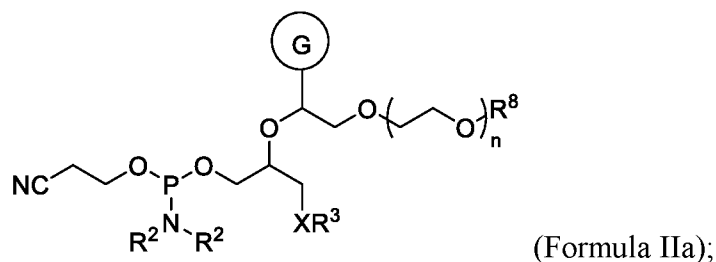


[0007] In some instances, Ring G is , , or , and each R⁴ is independently hydrogen, or substituted or unsubstituted benzoyl. In some instances,

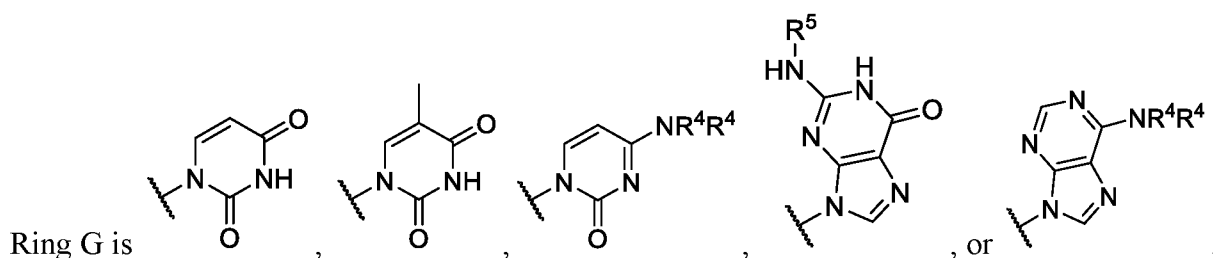


[0008] In some embodiments, each R² is independently hydrogen, deuterium, or substituted or unsubstituted C₁-C₁₀ alkyl. In some embodiments, each R² is independently substituted or unsubstituted C₁-C₁₀ alkyl. In some embodiments, each R² is independently isopropyl. In some embodiments, two R² are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C₃-C₁₀ heterocycloalkyl. In some embodiments, X is O, and R³ is 4,4'-dimethoxytrityl (DMT).

[0009] In certain embodiments, described herein is a compound according to Formula (IIa):



wherein:



X is O, NH, or C(=O);

n is 0, 1, 2, 3, or 4;

R⁸ is substituted or unsubstituted C₁-C₆ alkyl;

each R² is independently hydrogen, deuterium, or substituted or unsubstituted C₁-C₁₀ alkyl;

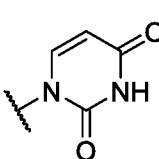
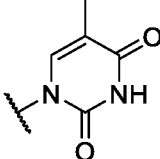
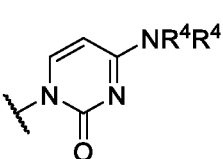
or two R² are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C₃-C₁₀cycloalkyl;

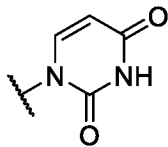
R³ is hydrogen, 4,4'-dimethoxytrityl (DMT), -R⁶, -OR⁶, or -NR⁷R⁷.

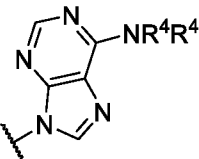
each R^4 is independently hydrogen, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted benzoyl;

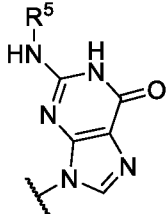
R^5 is $-C(=O)R^6$;

each R^6 and R^7 are independently hydrogen, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_1 - C_6 fluoroalkyl, substituted or unsubstituted C_3 - C_6 cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

[0010] In some instances, Ring G is , , or , and each R^4 is independently hydrogen, acetyl isobutyl or substituted or unsubstituted benzoyl. In

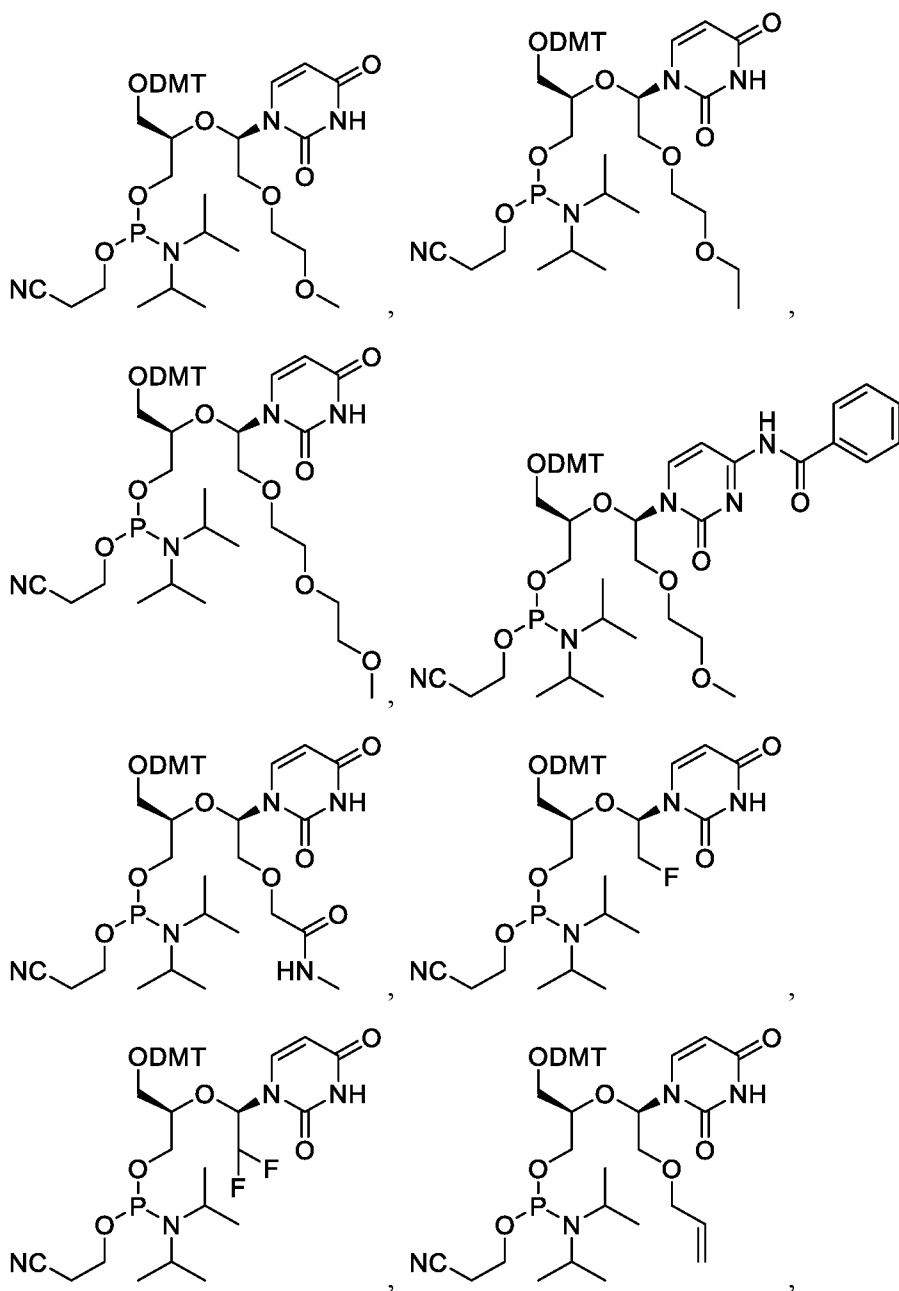
some instances, Ring G is . In some instances, Ring G is  or

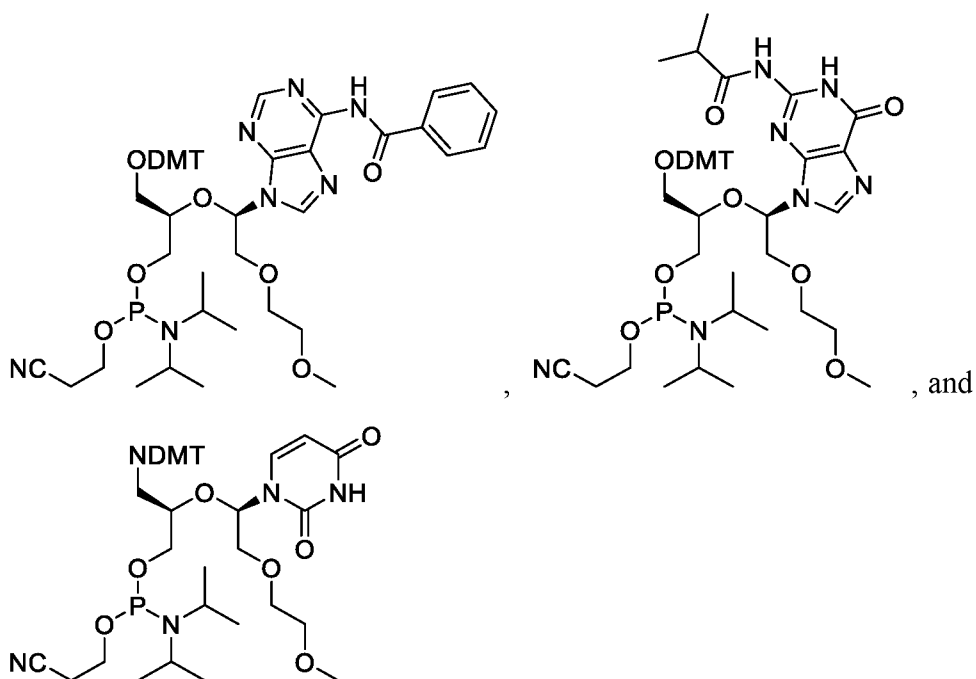
, and each R^4 is independently hydrogen or substituted or unsubstituted benzyl;

and R^5 is $-C(=O)R^6$. In some instances, Ring G is , and R^5 is $-C(=O)CH(CH_3)_2$.

[0011] In some instances, R^6 is substituted or unsubstituted C_1 - C_6 alkyl. Alternatively and/or additionally, R^8 is methyl or ethyl. Alternatively and/or additionally, n is 1 or 2. In some embodiments, n is 1. In some embodiments, each R^2 is independently hydrogen, deuterium, or substituted or unsubstituted C_1 - C_{10} alkyl. In some embodiments, each R^2 is independently substituted or unsubstituted C_1 - C_{10} alkyl. In some embodiments, each R^2 is independently isopropyl. In some embodiments, two R^2 are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C_3 - C_{10} heterocycloalkyl. In some instances, R^3 is 4,4'-dimethoxytrityl (DMT).

[0012] In some instances, the compound is selected from

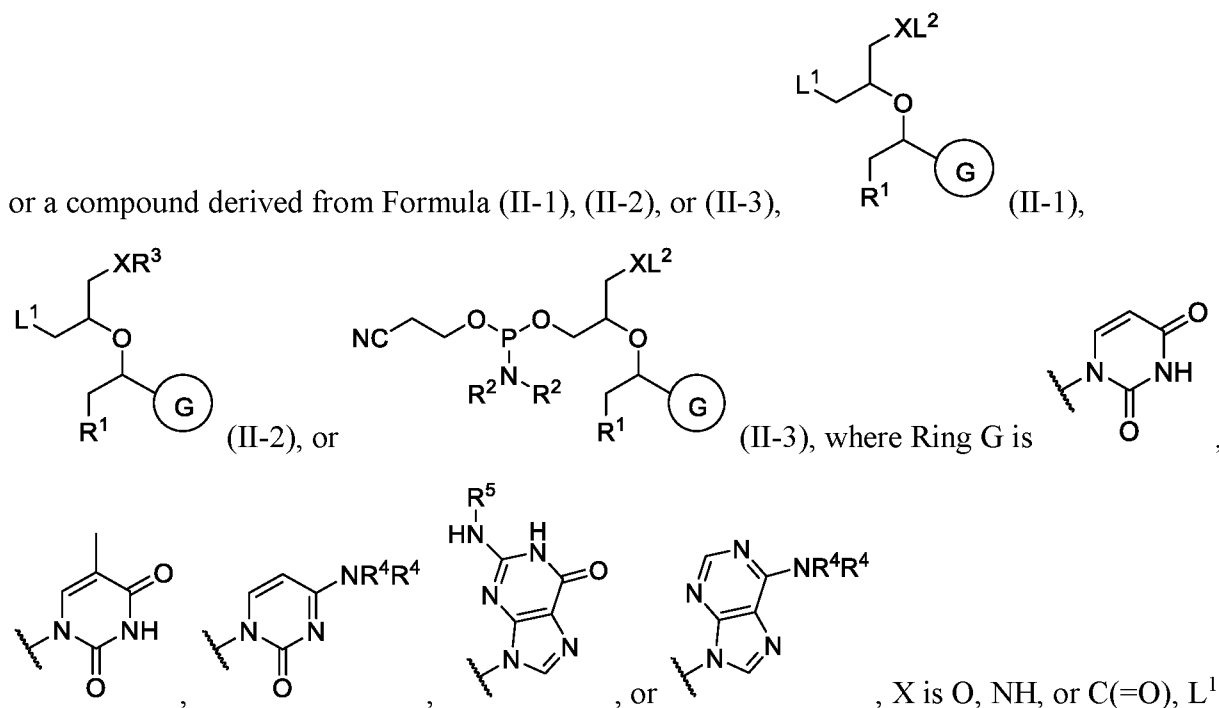




[0013] In some embodiments, the compound is suitable for the synthesis of oligonucleotides.

[0014] In certain embodiments, disclosed herein is an oligonucleotide comprising a compound of Formula (II) or Formula (IIa).

[0015] Disclosed herein, in certain embodiments, are compositions and pharmaceutical formulations that comprise a binding moiety conjugated to a polynucleic acid molecule and optionally a polymer. Thus, disclosed herein includes an oligonucleotide conjugate of Formula (I): A-B, where A is a binding moiety, and B is an oligonucleotide comprising a compound of,



and L^2 are each independently absent or a linking moiety selected from a phosphodiester, amide and ester linkage, R^1 is hydrogen, deuterium, halogen, substituted or unsubstituted C_1 - C_{10} alkyl,

substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, substituted or unsubstituted C₁-C₁₀ fluoroalkyl, substituted or unsubstituted C₁-C₂₀ heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted -O-aryl, -OR⁶, -C(=O)R⁶, -C(=O)OR⁶, -C(=O)NR⁷R⁷, -OCH₂C(=O)R⁶, -OCH₂C(=O)OR⁶, or -OCH₂C(=O)NR⁷R⁷, each R² is independently hydrogen, deuterium, or substituted or unsubstituted C₁-C₁₀ alkyl, or two R² are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C₃-C₁₀ heterocycloalkyl, R³ is hydrogen, 4,4'-dimethoxytrityl (DMT), -R⁶, -OR⁶, or -NR⁷R⁷, each R⁴ is independently hydrogen, substituted or unsubstituted C₁-C₆alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted benzoyl, R⁵ is -C(=O)OR⁶, and each R⁶ and R⁷ are independently hydrogen, substituted or unsubstituted C₁-C₆alkyl, substituted or unsubstituted C₁-C₆fluoroalkyl, substituted or unsubstituted C₃-C₆cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl. In some embodiments, a compound derived from a formula (e.g., a derivative compound from Formula II-3) comprises a modified form of the formula with a substituted group. For example, in one embodiment, a compound derived from Formula II-3 has a phosphodiester group, phosphotriester group or a phosphorothioate group substituting phosphonamidite group.

[0016] In some embodiments, the oligonucleotide is an RNA oligonucleotide. In some embodiments, the oligonucleotide comprises at least one modification, or at least one 2' modified nucleotide, which is selected from 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide, or alternatively selected from locked nucleic acid (LNA) or ethylene nucleic acid (ENA). Alternatively and/or additionally, the oligonucleotide comprises at least one modified internucleotide linkage, optionally selected from a phosphorothioate, a phosphorodithioate, a methylphosphonate, a phosphotriester, or an amide linkage.

[0017] In some embodiments, the derivative compound of Formula (II-3) is located at the 5'-terminus of the oligonucleotide. Alternatively and/or additionally, the compound of Formula (II-2) is located at the 3'-terminus of the oligonucleotide. Alternatively and/or additionally, the derivative compound of Formula (II-3) is located at the 5'-terminus of the oligonucleotide; and the binding moiety is conjugated to the 3'-terminus of the oligonucleotide. Alternatively and/or additionally, the compound of Formula (II-1) is located at an internal position within the oligonucleotide. Alternatively and/or additionally, the compound of Formula (II-2) is located at

the 5'-terminus of the oligonucleotide; and the binding moiety is conjugated to the 3'-terminus of the oligonucleotide. Alternatively and/or additionally, the compound of Formula (II-2) is located at the 3'-terminus of the oligonucleotide, and the binding moiety is conjugated to the 5'-terminus of the oligonucleotide. Alternatively and/or additionally, the compound of Formula (II-1) is located in the interior of the of the oligonucleotide, and the binding moiety is conjugated to the 5'-terminus or the 3'-terminus of the oligonucleotide. In some embodiments, Formula (I) further comprises C to form A-B-C (Formula I-A), wherein A and C are not attached to B at the same terminus. In some embodiments, C is a polymer, which can comprise polyethylene glycol.

[0018] In some embodiments, the binding moiety comprises an antibody or a binding fragment thereof. In some embodiments, the binding moiety comprises a humanized antibody or binding fragment thereof, a chimeric antibody or binding fragment thereof, a monoclonal antibody or binding fragment thereof, a monovalent Fab', a divalent Fab2, a single-chain variable fragment (scFv), a diabody, a minibody, a nanobody, a single-domain antibody (sdAb), or a camelid antibody or binding fragment thereof. In some embodiments, the binding moiety comprises a peptide or a small molecule.

[0019] In some embodiments, the oligonucleotide comprises from about 8 to about 50 nucleotides, or from about 10 to about 30 nucleotides.

[0020] In some embodiments, B of Formula I comprises an RNA oligonucleotide, has a length from about 10 to about 30 nucleotides, comprises at least one 2' modified nucleotide, and comprises at least one modified internucleotide linkage. In some embodiments, B hybridizes to at least 8 contiguous bases of a target gene sequence. In some embodiments, B mediates RNA interference. In some embodiments, B is a sense strand. In some embodiments, B is hybridized with a second oligonucleotide to form a double-stranded oligonucleic acid molecule. In some embodiments, the second oligonucleotide is an antisense strand. In some embodiments, the second oligonucleotide is an RNA oligonucleotide. In some embodiments, the second oligonucleotide comprises at least one modification. In some embodiments, the second oligonucleotide comprises a compound of Formula (II) or a compound of Formula (IIa). In some embodiments, the second oligonucleotide comprises at least one 2' modified nucleotide. In some embodiments, the second oligonucleotide comprises at least one 2' modified nucleotide selected from 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide, or alternatively and/or additionally selected from locked nucleic acid (LNA) or ethylene nucleic acid (ENA).

[0021] In some embodiments, the second oligonucleotide comprises at least one modified internucleotide linkage. In some embodiments, the second oligonucleotide comprises at least one, or at least three modified internucleotide linkage selected from a phosphorothioate linkage or a phosphorodithioate linkage.

[0022] In some embodiments, the oligonucleotide is a sense strand, is an RNA oligonucleotide, is conjugated to a binding moiety, a polymer, or a combination thereof, is from about 10 to about 30 nucleotides, comprises at least one 2' modified nucleotide, and comprises at least one modified internucleotide linkage.

[0023] In some embodiments, also described herein include methods for treating a disease or condition (e.g., cancer) that utilize a composition or a pharmaceutical formulation comprising a binding moiety conjugated to a polynucleic acid molecule and optionally a polymer. Thus, disclosed herein includes a method of treating a subject having a disease or condition characterized with a defective protein expression, comprising administering to the subject an oligonucleotide conjugate described herein to modulate expression of a gene encoding the protein, thereby treating the disease or condition characterized with the defective protein expression. Also disclosed herein includes a method of treating a subject having a disease or condition characterized with an overexpressed protein expression, comprising administering to the subject an oligonucleotide conjugate as described herein to modulate expression of a gene encoding the protein, thereby treating the disease or condition characterized with the overexpressed protein expression. In some embodiments, the disease or condition is a cancer, a neuromuscular disease, a muscle dystrophy, a muscle atrophy, a muscle wasting, a genetic disease, a hereditary disease, or a cardiovascular disease.

[0024] Disclosed herein, in certain embodiments, is a method of inhibiting the expression of a target gene in a primary cell of a patient, comprising administering a molecule described above to the primary cell. In some embodiments, the method is an *in vivo* method. In some embodiments, the patient is a human.

[0025] Disclosed herein, in certain embodiments, is an immuno-oncology therapy comprising a molecule described above for the treatment of a disease or disorder in a patient in need thereof.

[0026] Disclosed herein, in certain embodiments, is a kit comprising a molecule described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The disclosure may be better

understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0028] **FIG. 1A** shows a cartoon representation of a polynucleotide duplex, comprising a compound of Formula (II) attached to the 5' end of the guide (antisense) strand, and further comprising a binding moiety A, which may be attached to the polynucleotide directly or via a linker, attached to the 5' end of the passenger (sense) strand.

[0029] **FIG. 1B** shows a cartoon representation of a polynucleotide duplex, comprising one or more compounds of Formula (II) attached to the 5' end of the guide (antisense) strand, and inserted into the antisense strand, and further comprising a binding moiety A, which may be attached to the polynucleotide directly or via a linker, attached to the 5' end of the passenger (sense) strand.

[0030] **FIG. 2** shows a cartoon representation of an exemplary compound of Formula (II) in an oligonucleotide.

[0031] **FIG. 3** shows a graph of MSTN knockdown in SJCRH30 cells *in vitro* using four different types of UNA siRNAs.

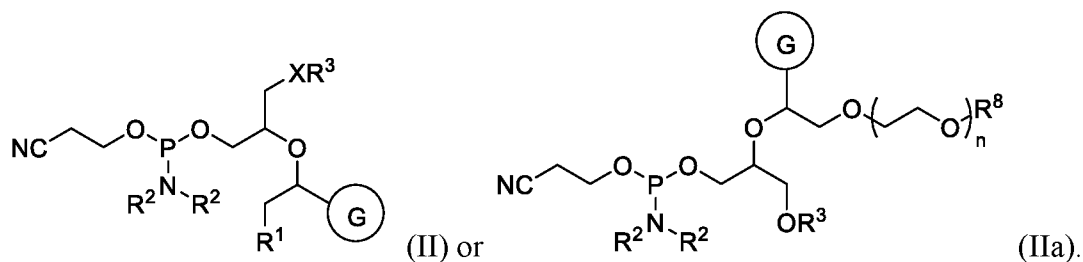
[0032] **FIG. 4** shows a graph of MSTN knockdown in Gastrocnemius tissue *in vivo* using α -CD71-UNA siRNA conjugates.

DETAILED DESCRIPTION

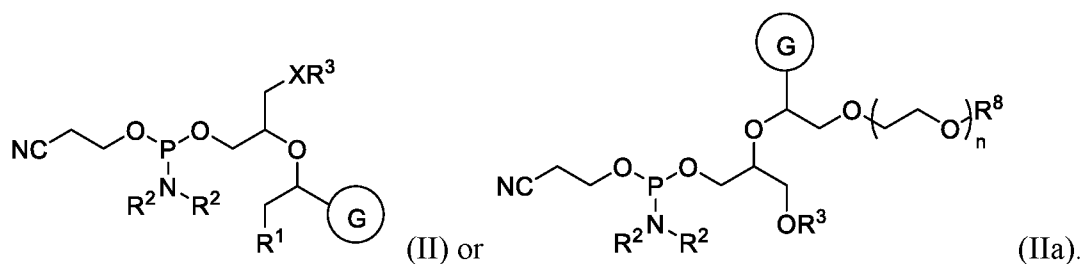
[0033] Nucleic acid (e.g., RNAi) therapy is a targeted therapy with high selectivity and specificity. However, in some instances, nucleic acid therapy is also hindered by poor intracellular uptake, limited blood stability and non-specific immune stimulation. To address these issues, various modifications of the nucleic acid composition are explored, such as for example, novel linkers for better stabilizing and/or lower toxicity, optimization of binding moiety for increased target specificity and/or target delivery, and nucleic acid polymer modifications for increased stability and/or reduced off-target effect.

[0034] In some embodiments, the arrangement or order of the different components that make-up the nucleic acid composition further effects intracellular uptake, stability, toxicity, efficacy, and/or non-specific immune stimulation. For example, if the nucleic acid component includes a binding moiety, a polymer, and a polynucleic acid molecule (or polynucleotide), the order or arrangement of the binding moiety, the polymer, and/or the polynucleic acid molecule (or polynucleotide) (e.g., binding moiety-polynucleic acid molecule-polymer, binding moiety-polymer-polynucleic acid molecule, or polymer-binding moiety-polynucleic acid molecule) further effects intracellular uptake, stability, toxicity, efficacy, and/or non-specific immune stimulation.

[0035] In some embodiments, described herein include an oligonucleotide conjugate whose arrangement of the nucleic acid components effects intracellular uptake, stability, toxicity, efficacy, and/or non-specific immune stimulation. In some instances, the oligonucleotide conjugate comprises a binding moiety conjugated to a polynucleic acid molecule and a polymer. In some embodiments, the oligonucleotide conjugate comprises a compound according to Formula (II) or Formula (IIa):



[0036] In some embodiments, an oligonucleotide conjugate comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer arranged as described herein enhances intracellular uptake, stability, and/or efficacy. In some instances, an oligonucleotide conjugate comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer arranged as described herein reduces toxicity and/or non-specific immune stimulation. In some cases, the oligonucleotide conjugate comprises a compound according to Formula (II) or Formula (IIa):



[0037] In additional embodiments, described herein include a kit, which comprises one or more of the molecules or compositions described herein.

Therapeutic Molecule Platform

[0038] In some embodiments, an oligonucleotide conjugate (e.g., a therapeutic oligonucleotide conjugate) described herein comprises a binding moiety conjugated to a polynucleic acid molecule comprising one or more modified nucleotides and a polymer. In some embodiments, the oligonucleotide conjugate comprises a compound according to Formula (I) or Formula (I-A):

A-B

Formula (I);

wherein,

A is a binding moiety; and

B is an oligonucleotide comprising a compound of Formula (II) or (IIa)

A-B-C

Formula (I-A);

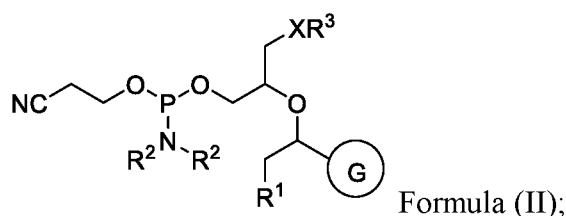
wherein,

A is a binding moiety; and

B is an oligonucleotide comprising a compound of Formula (II) or (IIa)

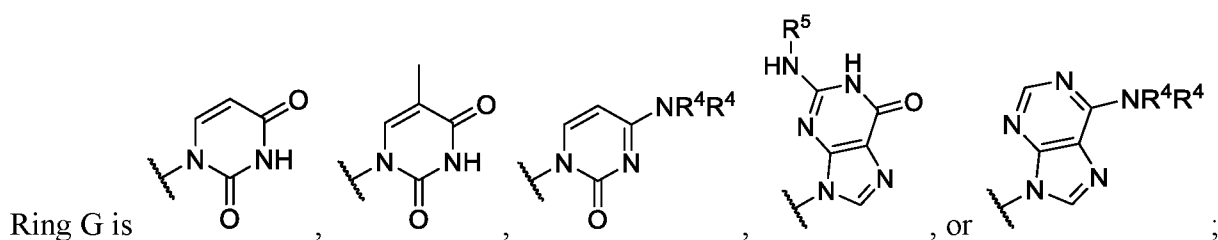
C is optionally a polymer.

[0039] In some embodiments, the oligonucleotide comprises a compound according to Formula (II):



Formula (II);

wherein,



X is O, NH, or C(=O);

R¹ is hydrogen, deuterium, halogen, substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, substituted or unsubstituted C₁-C₁₀ fluoroalkyl, substituted or unsubstituted C₁-C₂₀ heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted -O-aryl, -OR⁶, -C(=O)R⁶, -C(=O)OR⁶, -C(=O)NR⁷R⁷, -OCH₂C(=O)R⁶, -OCH₂C(=O)OR⁶, or -OCH₂C(=O)NR⁷R⁷;

each R² is independently hydrogen, deuterium, or substituted or unsubstituted C₁-C₁₀ alkyl; or two R² are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C₃-C₁₀ heterocycloalkyl;

R³ is hydrogen, 4,4'-dimethoxytrityl (DMT), -R⁶, -OR⁶, or -NR⁷R⁷;

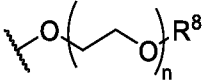
each R^4 is independently hydrogen, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted benzoyl;

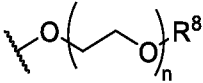
R^5 is $-C(=O)R^6$;

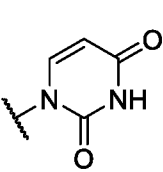
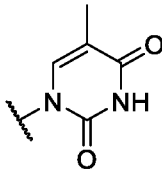
each R^6 and R^7 are independently hydrogen, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_1 - C_6 fluoroalkyl, substituted or unsubstituted C_3 - C_6 cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

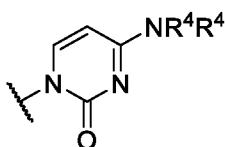
[0040] In some embodiments of Formula (II), R^1 is halogen, substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_1 - C_{10} fluoroalkyl, substituted or unsubstituted C_1 - C_{20} heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted $-O$ -aryl, $-OH$, $-OR^6$, $-C(=O)R^6$, $-C(=O)OR^6$, $-C(=O)NR^7R^7$, $-OCH_2C(=O)R^6$, or $-OCH_2C(=O)OR^6$, or $-OCH_2C(=O)NR^7R^7$.

[0041] In some embodiments of Formula (II), R^1 is halogen, substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_1 - C_{20} heteroalkyl, substituted or unsubstituted $-O$ -aryl, $-OCH_2C(=O)R^6$, or $-OCH_2C(=O)OR^6$, or $-OCH_2C(=O)NR^7R^7$. In some embodiments of Formula (II), R^1 is halogen or substituted or unsubstituted C_1 - C_{20} heteroalkyl. In some embodiments of Formula (II), R^1 is fluoro, chloro, or bromo. In some embodiments of Formula (II), R^1 is fluoro.

[0042] In some embodiments of Formula (II), R^1 is ; n is 0, 1, 2, 3, 4, or 5; and R^8 is hydrogen or substituted or unsubstituted C_1 - C_3 alkyl. In some embodiments, n is 1, 2, or 3. In some embodiments, n is 1 or 2. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 0.

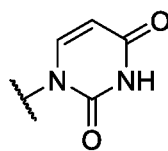
[0043] In some embodiments of Formula (II), R^1 is ; and R^8 is substituted or unsubstituted C_1 - C_3 alkyl. In some embodiments, R^8 is methyl or ethyl. In some embodiments, R^8 is methyl. In some embodiments, R^8 is ethyl. In some embodiments, R^8 is H.

[0044] In some embodiments of Formula (II), Ring G is , , or



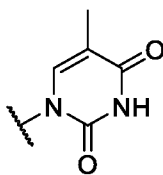
; wherein each R^4 is independently hydrogen or substituted or unsubstituted

benzoyl. In some embodiments of Formula (II), ring B is

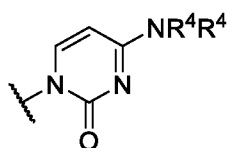


. In some embodiments

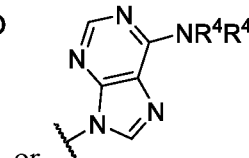
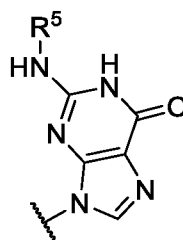
of Formula (II), Ring G is



. In some embodiments of Formula (II), Ring G is



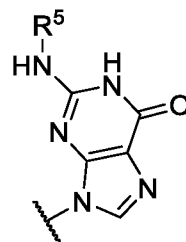
[0045] In some embodiments of Formula (II), Ring G is,



or ;

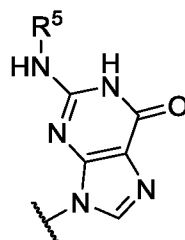
wherein each R^4 is independently hydrogen or substituted or unsubstituted benzoyl; and R^5 is -

$C(=O)R^6$. In some embodiments of Formula (II), Ring G is,



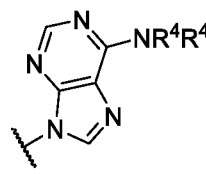
; wherein R^5 is -

$C(=O)R^6$. In some embodiments of Formula (II), Ring G is



; wherein R^5 is -

$C(=O)CH(CH_3)_2$. In some embodiments of Formula (II), Ring G is



; wherein

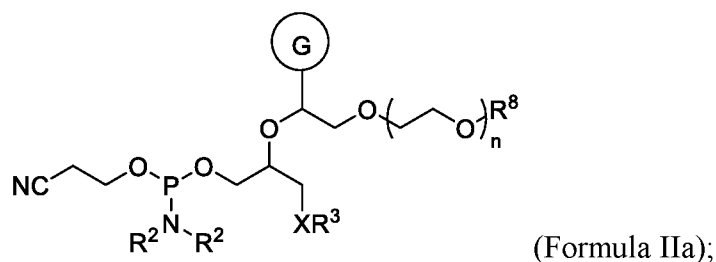
each R^4 is independently hydrogen or substituted or unsubstituted benzoyl.

[0046] In some embodiments of Formula (II), each R^2 is independently hydrogen, deuterium, or substituted or unsubstituted C_1 - C_{10} alkyl. In some embodiments of Formula (II), each R^2 is independently substituted or unsubstituted C_1 - C_{10} alkyl. In some embodiments of Formula (II), each R^2 is isopropyl.

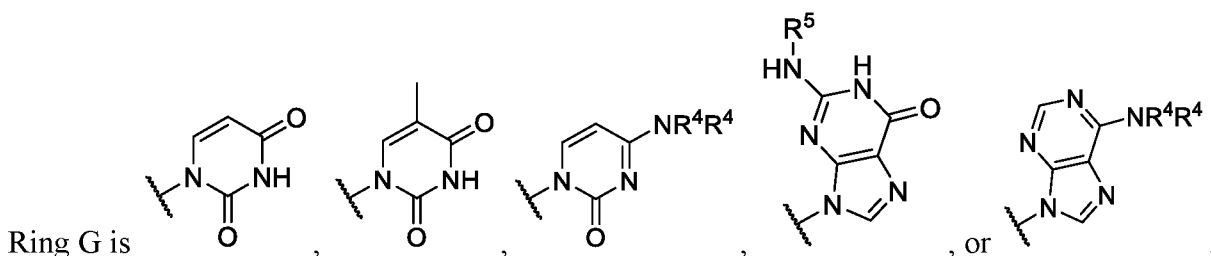
[0047] In some embodiments of Formula (II), two R^2 are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C_3 - C_{10} heterocycloalkyl.

[0048] In some embodiments of Formula (II), X is O and R^3 is 4,4'-dimethoxytrityl (DMT).

[0049] In some embodiments, the oligonucleotide comprises a compound according to Formula (IIa):



wherein:



X is O, NH, or C(=O);

n is 0, 1, 2, 3, or 4;

R^8 is substituted or unsubstituted C_1 - C_6 alkyl;

each R^2 is independently hydrogen, deuterium, or substituted or unsubstituted C_1 - C_{10} alkyl;

or two R^2 are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C_3 - C_{10} cycloalkyl;

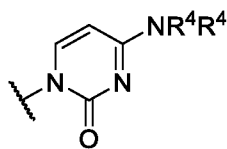
R^3 is hydrogen, 4,4'-dimethoxytrityl (DMT), $-R^6$, $-OR^6$, or $-NR^7R^7$.

each R^4 is independently hydrogen, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted benzoyl;

R^5 is $-C(=O)R^6$;

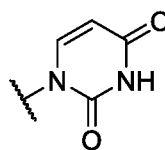
each R^6 and R^7 are independently hydrogen, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_1 - C_6 fluoroalkyl, substituted or unsubstituted C_3 - C_6 cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

[0050] In some embodiments of Formula (IIa), Ring G is



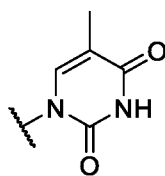
; wherein each R^4 is independently hydrogen or substituted or unsubstituted

benzyl. In some embodiments of Formula (IIa), Ring G is

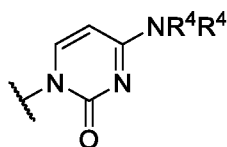


. In some embodiments

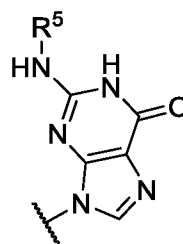
of Formula (II), Ring G is



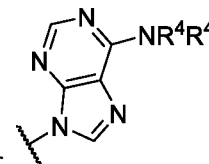
. In some embodiments of Formula (IIa), Ring G is



[0051] In some embodiments of Formula (IIa), Ring G is

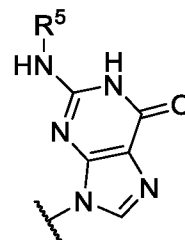


or



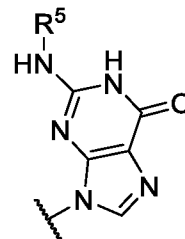
wherein each R^4 is independently hydrogen or substituted or unsubstituted benzyl; and R^5 is -

$C(=O)R^6$. In some embodiments of Formula (IIa), Ring G is

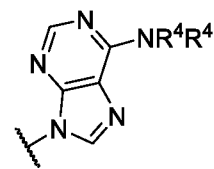


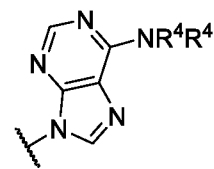
; wherein R^5 is -

$C(=O)R^6$. In some embodiments of Formula (IIa), Ring G is



; wherein R^5 is -



$C(=O)CH(CH_3)_2$. In some embodiments of Formula (IIa), Ring G is ; wherein each R^4 is independently hydrogen or substituted or unsubstituted benzoyl.

[0052] In some embodiments of Formula (IIa), n is 0, 1, 2, 3, 4, or 5. In some embodiments of Formula (IIa), n is 0, 1, 2, 3, or 4. In some embodiments of Formula (IIa), n is 1 or 2. In some embodiments of Formula (IIa), n is 0. In some embodiments of Formula (IIa), n is 1. In some embodiments of Formula (IIa), n is 2.

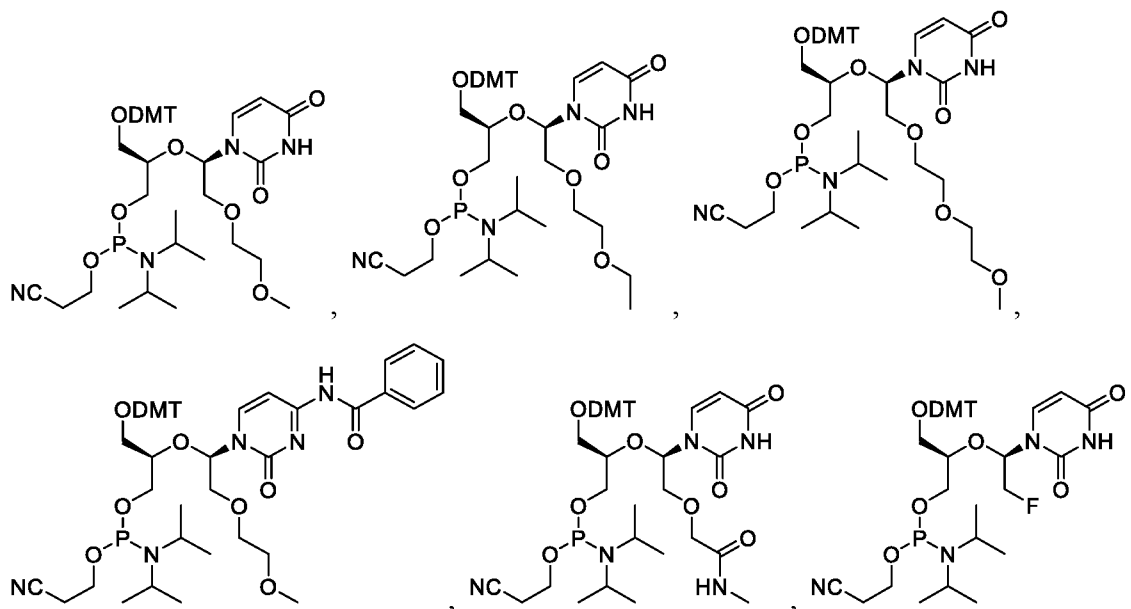
[0053] In some embodiments of Formula (IIa), R^8 is hydrogen or substituted or unsubstituted C_1 - C_3 alkyl. In some embodiments of Formula (IIa), R^8 is substituted or unsubstituted C_1 - C_3 alkyl. In some embodiments of Formula (IIa), R^8 is methyl or ethyl. In some embodiments of Formula (IIa), R^8 is methyl.

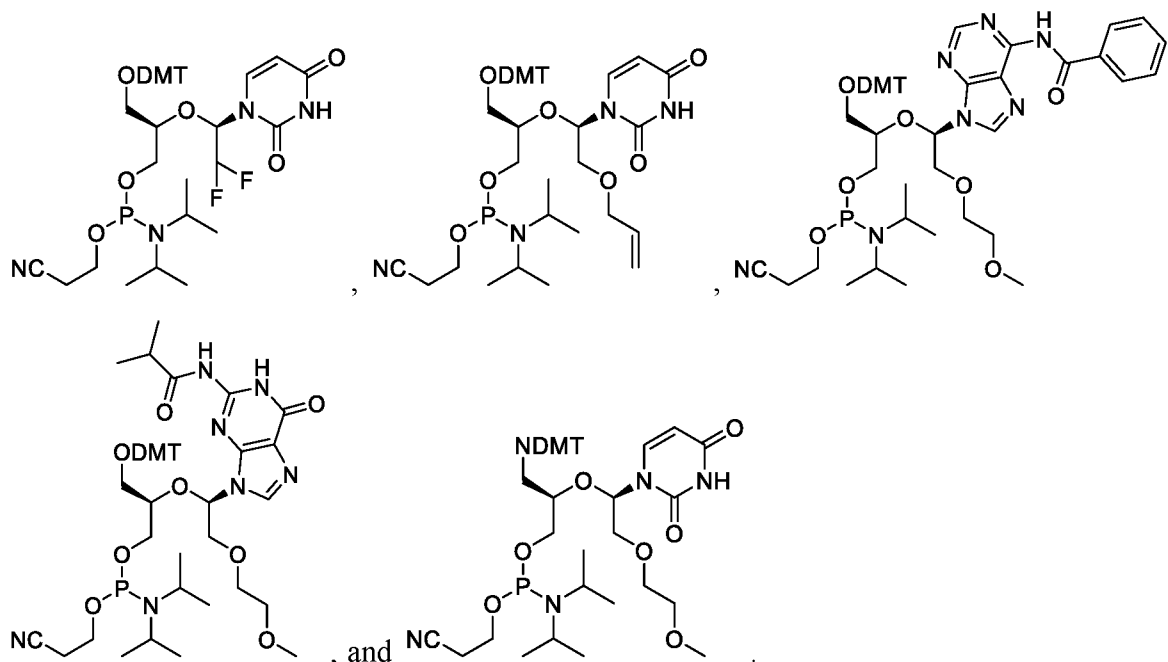
[0054] In some embodiments of Formula (IIa), each R^2 is independently hydrogen, deuterium, or substituted or unsubstituted C_1 - C_{10} alkyl. In some embodiments of Formula (IIa), each R^2 is independently substituted or unsubstituted C_1 - C_{10} alkyl. In some embodiments of Formula (IIa), each R^2 is isopropyl.

[0055] In some embodiments of Formula (IIa), two R^2 are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C_3 - C_{10} heterocycloalkyl.

[0056] In some embodiments of Formula (IIa), R^3 is 4,4'-dimethoxytrityl (DMT).

[0057] In some embodiments of Formula (II) or (IIa), the compound is selected from the group consisting of:

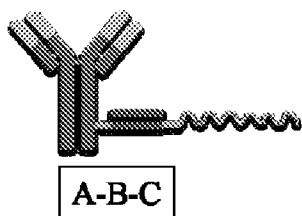




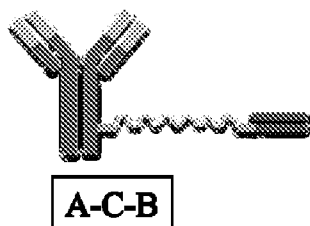
[0058] In some embodiments, the oligonucleotide comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 30, or more compounds of Formula (II) (e.g., Formula IIa). In some cases, the oligonucleotide comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, or more compounds of Formula (II) (e.g., Formula IIa). In some instances, the compounds of Formula (II) (e.g., Formula IIa) are in tandem within the oligonucleotide. In other instances, the compounds of Formula (II) (e.g., Formula IIa) are interspersed within the oligonucleotide, with nucleotides modified by one or more additional modification described below.

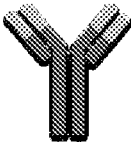
[0059] In some instances, the oligonucleotide comprises at least one of: from about 5% to about 100% modification, from about 10% to about 100% modification, from about 20% to about 100% modification, from about 30% to about 100% modification, from about 40% to about 100% modification, from about 50% to about 100% modification, from about 60% to about 100% modification, from about 70% to about 100% modification, from about 80% to about 100% modification, and from about 90% to about 100% modification, in which the modification is a compound of Formula (II) (e.g., Formula IIa). For example, where the oligonucleotide has 20 nucleosides, the oligonucleotide having about 60% modification comprises about 12 nucleosides substituted with 12 Formula (II) (e.g., Formula IIa) compounds.

[0060] In some embodiments, an oligonucleotide conjugate is a molecule as illustrated:



[0061] In some embodiments, an oligonucleotide conjugate is a molecule as illustrated:



[0062] The  as illustrated above is for representation purposes only and encompasses a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, anti-human antibody, anti-murine antibody (e.g., anti-mouse antibody, anti-rat antibody, etc.), monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, nanobody, single-domain antibody (sdAb), or camelid antibody or binding fragment thereof.

Additional Modifications

[0063] In some embodiments, the additional modifications include synthetic or artificial nucleotide analogues or bases comprising modifications at one or more of ribose moiety, phosphate moiety, nucleoside moiety, or a combination thereof.

[0064] In some embodiments, a modification at a 2' hydroxyl group include 2'-deoxy, 2-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA).

[0065] In some embodiments, a nucleotide analogue comprises a modified base such as, but not limited to, 5-propynyluridine, 5-propynylcytidine, 6-methyladenine, 6-methylguanine, N, N, -dimethyladenine, 2-propyladenine, 2-propylguanine, 2-aminoadenine, 1-methylinosine, 3-methyluridine, 5-methylcytidine, 5-methyluridine and other nucleotides having a modification at the 5 position, 5- (2- amino) propyl uridine, 5-halocytidine, 5-halouridine, 4-acetylcytidine, 1-methyladenosine, 2-methyladenosine, 3-methylcytidine, 6-methyluridine, 2-methylguanosine,

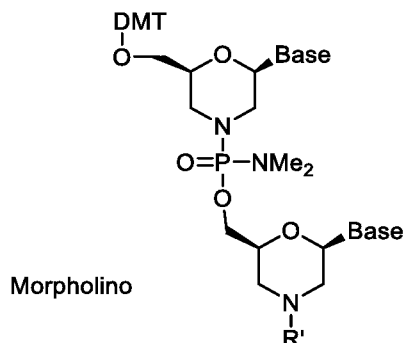
7-methylguanosine, 2, 2-dimethylguanosine, 5- methylaminoethyluridine, 5-methyloxyuridine, deazanucleotides (such as 7-deaza- adenosine, 6-azouridine, 6-azocytidine, or 6-azothymidine), 5-methyl-2-thiouridine, other thio bases (such as 2-thiouridine, 4-thiouridine, and 2-thiocytidine), dihydrouridine, pseudouridine, queuosine, archaeosine, naphthyl and substituted naphthyl groups, any O-and N-alkylated purines and pyrimidines (such as N6-methyladenosine, 5-methylcarbonylmethyluridine, uridine 5-oxyacetic acid, pyridine-4-one, or pyridine-2-one), phenyl and modified phenyl groups such as aminophenol or 2,4, 6-trimethoxy benzene, modified cytosines that act as G-clamp nucleotides, 8-substituted adenines and guanines, 5-substituted uracils and thymines, azapyrimidines, carboxyhydroxyalkyl nucleotides, carboxyalkylaminoalkyl nucleotides, and alkylcarbonylalkylated nucleotides. 5'-modified nucleotides also include those nucleotides that are modified with respect to the sugar moiety, as well as 5'-modified nucleotides having sugars or analogs thereof that are not ribosyl. For example, the sugar moieties, in some cases are or are based on, mannoses, arabinoses, glucopyranoses, galactopyranoses, 4'-thioribose, and other sugars, heterocycles, or carbocycles. The term nucleotide also includes what are known in the art as universal bases. By way of example, universal bases include but are not limited to 3-nitropyrrole, 5-nitroindole, or nebularine.

[0066] In some instances, the modification at the 2' hydroxyl group is a 2'-O-aminopropyl modification in which an extended amine group comprising a propyl linker binds the amine group to the 2' oxygen. In some instances, this modification neutralizes the phosphate-derived overall negative charge of the oligonucleotide molecule by introducing one positive charge from the amine group per sugar and thereby improves cellular uptake properties due to its zwitterionic properties.

[0067] In some instances, the 5'-modified nucleotide is further modified at the 2' hydroxyl group in a locked or bridged ribose modification (e.g., locked nucleic acid or LNA) in which the oxygen molecule bound at the 2' carbon is linked to the 4' carbon by a methylene group, thus forming a 2'-C,4'-C-oxy-methylene-linked bicyclic ribonucleotide monomer.

[0068] In some embodiments, the additional modification further comprises a morpholino, a peptide nucleic acid (PNA), a methylphosphonate nucleotide, a thiophosphonate nucleotide, a 2'-fluoro N3-P5'-phosphoramidite, or a 1', 5'- anhydrohexitol nucleic acid (HNA). Morpholino or phosphorodiamidate morpholino oligo (PMO) comprises synthetic molecules whose structure mimics natural nucleic acid structure but deviates from the normal sugar and phosphate structures. In some instances, the five member ribose ring is substituted with a six member morpholino ring containing four carbons, one nitrogen, and one oxygen. In some cases, the ribose monomers are linked by a phosphordiamidate group instead of a phosphate group. In such

cases, the backbone alterations remove all positive and negative charges making morpholinos neutral molecules capable of crossing cellular membranes without the aid of cellular delivery agents such as those used by charged oligonucleotides. A non-limiting example of a 5'-modified morpholino oligonucleotide is illustrated below.

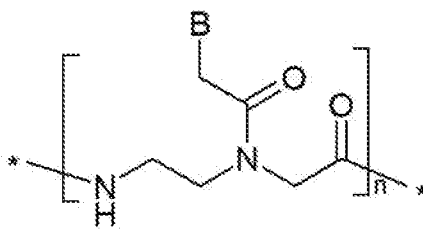


[0069] In some embodiments, a 5'-modified morpholino or PMO described above is a PMO comprising a positive or cationic charge. In some instances, the PMO is *PMOplus* (Sarepta). *PMOplus* refers to phosphorodiamidate morpholino oligomers comprising any number of (1-piperazino)phosphinylideneoxy, (1-(4-(omega-guanidino-alkanoyl))-piperazino)phosphinylideneoxy linkages (e.g., as such those described in PCT Publication No. WO2008/036127. In some cases, the PMO is a PMO described in U.S. Patent No. 7943762.

[0070] In some embodiments, a morpholino or PMO described above is a PMO-X (Sarepta). In some cases, PMO-X refers to phosphorodiamidate morpholino oligomers comprising at least one linkage or at least one of the disclosed terminal modifications, such as those disclosed in PCT Publication No. WO2011/150408 and U.S. Publication No. 2012/0065169.

[0071] In some embodiments, a morpholino or PMO described above is a PMO as described in Table 5 of U.S. Publication No. 2014/0296321.

[0072] In some embodiments, peptide nucleic acid (PNA) does not contain sugar ring or phosphate linkage and the bases are attached and appropriately spaced by oligoglycine-like molecules, therefore, eliminating a backbone charge.

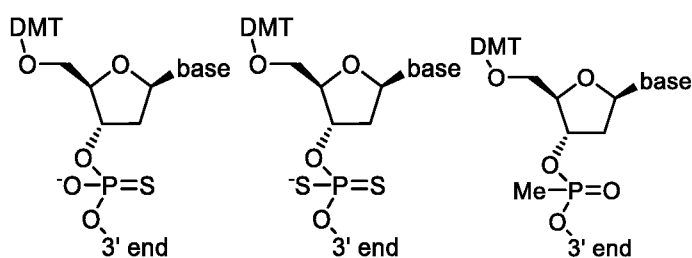


PNA

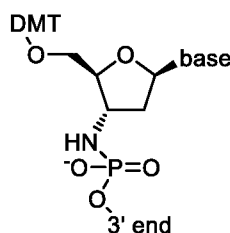
[0073] In some embodiments, one or more modifications described above occur at the internucleotide linkage. In some instances, modified internucleotide linkage includes, but is not limited to, phosphorothioates; phosphorodithioates; methylphosphonates; 5'-

alkylenephosphonates; 5'-methylphosphonate; 3'-alkylene phosphonates; borontrifluoridates; borano phosphate esters and selenophosphates of 3'-5'linkage or 2'-5'linkage; phosphotriesters; thionoalkylphosphotriesters; hydrogen phosphonate linkages; alkyl phosphonates; alkylphosphonothioates; arylphosphonothioates; phosphoroselenoates; phosphorodiselenoates; phosphinates; phosphoramidates; 3'- alkylphosphoramidates; aminoalkylphosphoramidates; thionophosphoramidates; phosphoropiperazidates; phosphoroanilothioates; phosphoroanilidates; ketones; sulfones; sulfonamides; carbonates; carbamates; methylenehydrazos; methylenedimethylhydrazos; formacetals; thioformacetals; oximes; methyleneiminos; methylenemethyliminos; thioamidates; linkages with riboacetyl groups; aminoethyl glycine; silyl or siloxane linkages; alkyl or cycloalkyl linkages with or without heteroatoms of, for example, 1 to 10 carbons that are saturated or unsaturated and/or substituted and/or contain heteroatoms; linkages with morpholino structures, amides, or polyamides wherein the bases are attached to the aza nitrogens of the backbone directly or indirectly; and combinations thereof.

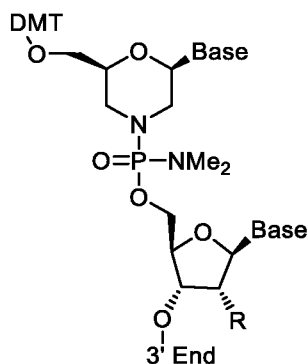
[0074] In some instances, the modification is a methyl or thiol modification such as methylphosphonate or thiophosphonate modification. Exemplary thiophosphonate nucleotide (left), phosphorodithioates (center) and methylphosphonate nucleotide (right) are illustrated below.



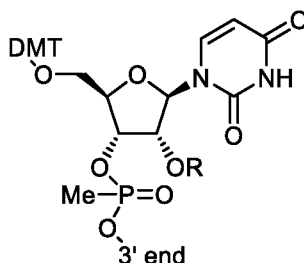
[0075] In some instances, a 5'-modified nucleotide includes, but is not limited to, phosphoramidites illustrated as:



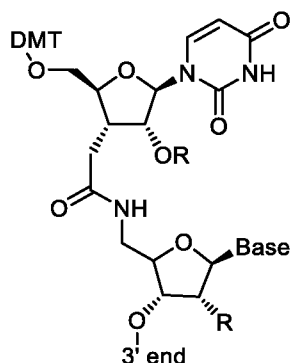
[0076] In some instances, the modified internucleotide linkage is a phosphorodiamidate linkage. A non-limiting example of a phosphorodiamidate linkage with a morpholino system is shown below.



[0077] In some instances, the modified internucleotide linkage is a methylphosphonate linkage. A non-limiting example of a methylphosphonate linkage is shown below.



[0078] In some instances, the modified internucleotide linkage is an amide linkage. A non-limiting example of an amide linkage is shown below.



[0079] In some embodiments, one or more modifications comprise a modified phosphate backbone in which the modification generates a neutral or uncharged backbone. In some instances, the phosphate backbone is modified by alkylation to generate an uncharged or neutral phosphate backbone. As used herein, alkylation includes methylation, ethylation, and propylation. In some cases, an alkyl group, as used herein in the context of alkylation, refers to a linear or branched saturated hydrocarbon group containing from 1 to 6 carbon atoms. In some instances, exemplary alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, hexyl, isohexyl, 1, 1-dimethylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, and 2-ethylbutyl groups. In some cases, a modified phosphate is a phosphate group as described in U.S. Patent No. 9481905.

[0080] In some embodiments, additional modified phosphate backbones comprise methylphosphonate, ethylphosphonate, methylthiophosphonate, or methoxyphosphonate. In some cases, the modified phosphate is methylphosphonate. In some cases, the modified phosphate is ethylphosphonate. In some cases, the modified phosphate is methylthiophosphonate. In some cases, the modified phosphate is methoxyphosphonate.

[0081] In some embodiments, one or more modifications further optionally include modifications of the ribose moiety, phosphate backbone and the nucleoside, or modifications of the nucleotide analogues at the 3' or the 5' terminus. For example, the 3' terminus optionally include a 3' cationic group, or by inverting the nucleoside at the 3'-terminus with a 3'-3' linkage. In another alternative, the 3'-terminus is optionally conjugated with an aminoalkyl group, e.g., a 3' C5-aminoalkyl dT. In an additional alternative, the 3'-terminus is optionally conjugated with an abasic site, e.g., with an apurinic or apyrimidinic site.

[0082] In some embodiments, the oligonucleotide comprising a compound of Formula (II) (e.g., Formula IIa) further comprises one or more of the artificial nucleotide analogues described herein. In some instances, the oligonucleotide comprising a compound of Formula (II) (e.g., Formula IIa) further comprises one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more additional modifications such as, but not limited to, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiophosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof. In some instances, the oligonucleotide comprising a compound of Formula (II) (e.g., Formula IIa) further comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of the artificial nucleotide analogues selected from 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiophosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof. In some instances, the oligonucleotide comprising a compound of Formula (II) (e.g., Formula IIa) further comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of 2'-O-methyl modified nucleotides. In some instances, the oligonucleotide comprising a compound of Formula (II) (e.g., Formula IIa) further comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of 2'-O-

methoxyethyl (2'-O-MOE) modified nucleotides. In some instances, the oligonucleotide comprising a compound of Formula (II) (e.g., Formula IIa) further comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of thiolphosphonate nucleotides.

[0083] In some instances, about 5 to about 100% of the oligonucleotide comprising a compound of Formula (II) (e.g., Formula IIa) comprise the artificial nucleotide analogues described herein. In some instances, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some embodiments, the artificial nucleotide analogues include 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof.

[0084] In some embodiments, the oligonucleotide (or B) described herein comprises RNA or DNA. In some cases, the oligonucleotide comprises RNA. In some instances, RNA comprises short interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA (miRNA), double-stranded RNA (dsRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), or heterogeneous nuclear RNA (hnRNA). In some instances, RNA comprises shRNA. In some instances, RNA comprises miRNA. In some instances, RNA comprises dsRNA. In some instances, RNA comprises tRNA. In some instances, RNA comprises rRNA. In some instances, RNA comprises hnRNA. In some instances, the RNA comprises siRNA. In some cases, the oligonucleotide comprises a sense strand (or passenger strand) of a siRNA. In other cases, the oligonucleotide comprises an antisense (or guide strand) of a siRNA.

[0085] In some embodiments, the oligonucleotide is from about 10 to about 50 nucleotides in length. In some instances, the oligonucleotide is from about 10 to about 30, from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length.

[0086] In some embodiments, the oligonucleotide is about 50 nucleotides in length. In some instances, the oligonucleotide is about 45 nucleotides in length. In some instances, the oligonucleotide is about 40 nucleotides in length. In some instances, the oligonucleotide is about 35 nucleotides in length. In some instances, the oligonucleotide is about 30 nucleotides in length. In some instances, the oligonucleotide is about 25 nucleotides in length. In some instances, the oligonucleotide is about 20 nucleotides in length. In some instances, the oligonucleotide is about 19 nucleotides in length. In some instances, the oligonucleotide is

about 18 nucleotides in length. In some instances, the oligonucleotide is about 17 nucleotides in length. In some instances, the oligonucleotide is about 16 nucleotides in length. In some instances, the oligonucleotide is about 15 nucleotides in length. In some instances, the oligonucleotide is about 14 nucleotides in length. In some instances, the oligonucleotide is about 13 nucleotides in length. In some instances, the oligonucleotide is about 12 nucleotides in length. In some instances, the oligonucleotide is about 11 nucleotides in length. In some instances, the oligonucleotide is about 10 nucleotides in length. In some instances, the oligonucleotide is from about 10 to about 50 nucleotides in length. In some instances, the oligonucleotide is from about 10 to about 45 nucleotides in length. In some instances, the oligonucleotide is from about 10 to about 40 nucleotides in length. In some instances, the oligonucleotide is from about 10 to about 35 nucleotides in length. In some instances, the oligonucleotide is from about 10 to about 30 nucleotides in length. In some instances, the oligonucleotide is from about 10 to about 25 nucleotides in length. In some instances, the oligonucleotide is from about 10 to about 20 nucleotides in length. In some instances, the oligonucleotide is from about 15 to about 25 nucleotides in length. In some instances, the oligonucleotide is from about 19 to about 23 nucleotides in length. In some instances, the oligonucleotide is from about 15 to about 30 nucleotides in length. In some instances, the oligonucleotide is from about 12 to about 30 nucleotides in length.

[0087] In some embodiments, the oligonucleotide is further hybridized with a second oligonucleotide to form a duplex. In some instances, the oligonucleotide is a sense strand or passenger strand. In some instances, the second oligonucleotide is an antisense strand or guide strand.

[0088] In some embodiments, the second oligonucleotide is from about 10 to about 50 nucleotides in length. In some instances, the second oligonucleotide is from about 10 to about 30, from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length.

[0089] In some instances, the second oligonucleotide is about 50 nucleotides in length. In some instances, the second oligonucleotide is about 45 nucleotides in length. In some instances, the second oligonucleotide is about 40 nucleotides in length. In some instances, the second oligonucleotide is about 35 nucleotides in length. In some instances, the second oligonucleotide is about 30 nucleotides in length. In some instances, the second oligonucleotide is about 25 nucleotides in length. In some instances, the second oligonucleotide is about 20 nucleotides in length. In some instances, the second oligonucleotide is about 19 nucleotides in length. In some instances, the second oligonucleotide is about 18 nucleotides in length. In some instances, the second oligonucleotide is about 17 nucleotides in length. In some instances, the second

oligonucleotide is about 16 nucleotides in length. In some instances, the second oligonucleotide is about 15 nucleotides in length. In some instances, the second oligonucleotide is about 14 nucleotides in length. In some instances, the second oligonucleotide is about 13 nucleotides in length. In some instances, the second oligonucleotide is about 12 nucleotides in length. In some instances, the second oligonucleotide is about 11 nucleotides in length. In some instances, the second oligonucleotide is about 10 nucleotides in length. In some instances, the second oligonucleotide is from about 10 to about 50 nucleotides in length. In some instances, the second oligonucleotide is from about 10 to about 45 nucleotides in length. In some instances, the second oligonucleotide is from about 10 to about 40 nucleotides in length. In some instances, the second oligonucleotide is from about 10 to about 35 nucleotides in length. In some instances, the second oligonucleotide is from about 10 to about 30 nucleotides in length. In some instances, the second oligonucleotide is from about 10 to about 25 nucleotides in length. In some instances, the second oligonucleotide is from about 10 to about 20 nucleotides in length. In some instances, the second oligonucleotide is from about 15 to about 25 nucleotides in length. In some instances, the second oligonucleotide is from about 15 to about 25 nucleotides in length. In some instances, the second oligonucleotide is from about 15 to about 30 nucleotides in length. In some instances, the second oligonucleotide is from about 12 to about 30 nucleotides in length.

[0090] In some embodiments, a polynucleic acid molecule comprises a first oligonucleotide and a second oligonucleotide. In some instances, the polynucleic acid molecule further comprises a blunt terminus, an overhang, or a combination thereof. In some instances, the blunt terminus is a 5' blunt terminus, a 3' blunt terminus, or both. In some cases, the overhang is a 5' overhang, 3' overhang, or both. In some cases, the overhang comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-base pairing nucleotides. In some cases, the overhang comprises 1, 2, 3, 4, 5, or 6 non-base pairing nucleotides. In some cases, the overhang comprises 1, 2, 3, or 4 non-base pairing nucleotides. In some cases, the overhang comprises 1 non-base pairing nucleotide. In some cases, the overhang comprises 2 non-base pairing nucleotides. In some cases, the overhang comprises 3 non-base pairing nucleotides. In some cases, the overhang comprises 4 non-base pairing nucleotides.

[0091] In some embodiments, the sequence of the polynucleic acid molecule is at least 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% complementary to a target sequence described herein. Exemplary target sequence includes, but not limited to, any sequences of DMD gene or its mRNA, DMPK gene or its mRNA, any sequences of an oncogene or its mRNA, any genes related to hereditary or genetic diseases (e.g., GYS1), any sequences of a gene related to muscle atrophy, muscle dystrophy, or muscle wasting (e.g., DMD, DMPK, DUX4) and its mRNA. In some embodiments, the sequence of the polynucleic acid molecule is at least 50%

complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 60% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 70% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 80% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 90% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 95% complementary to a target sequence described herein. In some instances, the sequence of the polynucleic acid molecule is 100% complementary to a target sequence described herein.

[0092] In some embodiments, the sequence of the polynucleic acid molecule has 5 or less mismatches to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule has 4 or less mismatches to a target sequence described herein. In some instances, the sequence of the polynucleic acid molecule has 3 or less mismatches to a target sequence described herein. In some cases, the sequence of the polynucleic acid molecule has 2 or less mismatches to a target sequence described herein. In some cases, the sequence of the polynucleic acid molecule has 1 or less mismatches to a target sequence described herein.

[0093] In some embodiments, the specificity of the polynucleic acid molecule that hybridizes to a target sequence described herein is a 95%, 98%, 99%, 99.5%, or 100% sequence complementarity of the polynucleic acid molecule to a target sequence. In some instances, the hybridization is a high stringent hybridization condition.

[0094] In some embodiments, the polynucleic acid molecule hybridizes to at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 8 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 9 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 10 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 11 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 12 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 13 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 14 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule

hybridizes to at least 15 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 16 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 17 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 18 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 19 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 20 contiguous bases of a target sequence described herein.

[0095] In some embodiments, the polynucleic acid molecule has reduced off-target effect. In some instances, “off-target” or “off-target effects” refer to any instance in which a polynucleic acid polymer directed against a given target causes an unintended effect by interacting either directly or indirectly with another mRNA sequence, a DNA sequence or a cellular protein or other moiety. In some instances, an “off-target effect” occurs when there is a simultaneous degradation of other transcripts due to partial homology or complementarity between that other transcript and the sense and/or antisense strand of the polynucleic acid molecule.

[0096] In some cases, one or more of the artificial nucleotide analogues described herein are resistant toward nucleases such as for example ribonuclease such as RNase H, deoxyribonuclease such as DNase, or exonuclease such as 5'-3' exonuclease and 3'-5' exonuclease when compared to natural polynucleic acid molecules. In some instances, artificial nucleotide analogues comprising a compound of Formula II (e.g., Formula IIa), 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or combinations thereof are resistant toward nucleases such as for example ribonuclease such as RNase H, deoxyribonuclease such as DNase, or exonuclease such as 5'-3' exonuclease and 3'-5' exonuclease. In some instances, 2'-O-methyl modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-O-methoxyethyl (2'-O-MOE) modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-O-aminopropyl modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-deoxy modified polynucleic acid molecule is nuclease resistant (e.g., RNase H,

DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-deoxy-2'-fluoro modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-O-aminopropyl (2'-O-AP) modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-O-dimethylaminoethyl (2'-O-DMAOE) modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-O-dimethylaminopropyl (2'-O-DMAP) modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE) modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-O-N-methylacetamido (2'-O-NMA) modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, LNA-modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, ENA-modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, HNA-modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). Morpholinos may be nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, PNA-modified polynucleic acid molecule is resistant to nucleases (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, methylphosphonate nucleotide-modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, thiolphosphonate nucleotide-modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, polynucleic acid molecule comprising 2'-fluoro N3-P5'-phosphoramidites is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, the 5' conjugates described herein inhibit 5'-3' exonucleolytic cleavage. In some instances, the 3' conjugates described herein inhibit 3'-5' exonucleolytic cleavage.

[0097] In some embodiments, one or more of the artificial nucleotide analogues comprising a compound of Formula II (e.g., Formula IIa), described herein have increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. The one or more of the artificial nucleotide analogues comprising a compound of Formula II (e.g., Formula IIa), 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-

dimethylaminopropyl (2'-O-DMAP), 2'-O- dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, or 2'-fluoro N3-P5'-phosphoramidites can have increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-methyl modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-methoxyethyl (2'-O-MOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-aminopropyl modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-deoxy modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-deoxy-2'-fluoro modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-aminopropyl (2'-O-AP) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-dimethylaminoethyl (2'-O-DMAOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-dimethylaminopropyl (2'-O-DMAP) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-N-methylacetamido (2'-O-NMA) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, LNA-modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, ENA-modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, PNA-modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, HNA-modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, morpholino-modified polynucleic acid molecule

has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, methylphosphonate nucleotide-modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, thiophosphonate nucleotide-modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, polynucleic acid molecule comprising 2'-fluoro N3-P5'-phosphoramidites has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some cases, the increased affinity is illustrated with a lower K_d, a higher melt temperature (T_m), or a combination thereof.

[0098] In some embodiments, an artificial nucleotide analogues comprising a compound of Formula II (e.g., Formula IIa), described herein is a chirally pure (or stereo pure) polynucleic acid molecule, or a polynucleic acid molecule comprising a single enantiomer. In some instances, the polynucleic acid molecule comprises L-nucleotide. In some instances, the polynucleic acid molecule comprises D-nucleotides. In some instance, a polynucleic acid molecule composition comprises less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or less of its mirror enantiomer. In some cases, a polynucleic acid molecule composition comprises less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or less of a racemic mixture. In some instances, the polynucleic acid molecule is a polynucleic acid molecule described in: U.S. Patent Publication Nos: 2014/194610 and 2015/211006; and PCT Publication No.: WO2015107425.

[0099] In some embodiments, a polynucleic acid molecule described herein is further modified to include an aptamer-conjugating moiety. In some instances, the aptamer conjugating moiety is a DNA aptamer-conjugating moiety. In some instances, the aptamer-conjugating moiety is Alphamer (Centauri Therapeutics), which comprises an aptamer portion that recognizes a specific cell-surface target and a portion that presents a specific epitopes for attaching to circulating antibodies. In some instance, a polynucleic acid molecule described herein is further modified to include an aptamer-conjugating moiety as described in: U.S. Patent Nos: 8,604,184, 8,591,910, and 7,850,975.

[0100] In additional embodiments, a polynucleic acid molecule described herein is modified to increase its stability. In some embodiment, the polynucleic acid molecule is RNA (e.g., siRNA), the polynucleic acid molecule is modified to increase its stability. In some instances, the polynucleic acid molecule is modified by one or more of the modifications described above to increase its stability. In some cases, the polynucleic acid molecule is modified at the 2' hydroxyl position, such as by 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy,

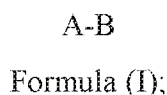
2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modification or by a locked or bridged ribose conformation (e.g., LNA or ENA). In some cases, the polynucleic acid molecule is modified by 2'-O-methyl and/or 2'-O-methoxyethyl ribose. In some cases, the polynucleic acid molecule also includes morpholinos, PNAs, HNA, methylphosphonate nucleotides, thiolphosphonate nucleotides, and/or 2'-fluoro N3-P5'-phosphoramidites to increase its stability. In some instances, the polynucleic acid molecule is a chirally pure (or stereo pure) polynucleic acid molecule. In some instances, the chirally pure (or stereo pure) polynucleic acid molecule is modified to increase its stability. Suitable modifications to the RNA to increase stability for delivery will be apparent to the skilled person.

[0101] In some embodiments, a polynucleic acid molecule described herein has RNAi activity that modulates expression of RNA encoded by a gene described *supra*. In some instances, a polynucleic acid molecule described herein is a double-stranded siRNA molecule that down-regulates expression of a gene, wherein one of the strands of the double-stranded siRNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of the gene or RNA encoded by the gene or a portion thereof, and wherein the second strand of the double-stranded siRNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence of the gene or RNA encoded by the gene or a portion thereof. In some cases, a polynucleic acid molecule described herein is a double-stranded siRNA molecule that down-regulates expression of a gene, wherein each strand of the siRNA molecule comprises about 15 to 25, 18 to 24, or 19 to about 23 nucleotides, and wherein each strand comprises at least about 14, 17, or 19 nucleotides that are complementary to the nucleotides of the other strand. In some cases, a polynucleic acid molecule described herein is a double-stranded siRNA molecule that down-regulates expression of a gene, wherein each strand of the siRNA molecule comprises about 19 to about 23 nucleotides, and wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand.

[0102] In some embodiments, a polynucleic acid molecule described herein is constructed using chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. For example, a polynucleic acid molecule is chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the polynucleic acid molecule and target nucleic acids. Exemplary methods include those described in: U.S. Patent Nos. 5,142,047; 5,185,444; 5,889,136; 6,008,400; and 6,111,086; PCT Publication No. WO2009099942; or European Publication No. 1579015. Additional exemplary methods include

those described in: Griffey et al., "2'-O-aminopropyl ribonucleotides: a zwitterionic modification that enhances the exonuclease resistance and biological activity of antisense oligonucleotides," *J. Med. Chem.* **39**(26):5100-5109 (1997)); Obika, et al. "Synthesis of 2'-O,4'-C-methyleneuridine and -cytidine. Novel bicyclic nucleosides having a fixed C3, -endo sugar puckering". *Tetrahedron Letters* **38** (50): 8735 (1997); Koizumi, M. "ENA oligonucleotides as therapeutics". *Current opinion in molecular therapeutics* **8** (2): 144-149 (2006); and Abramova et al., "Novel oligonucleotide analogues based on morpholino nucleoside subunits-antisense technologies: new chemical possibilities," *Indian Journal of Chemistry* 48B:1721-1726 (2009). Alternatively, the polynucleic acid molecule is produced biologically using an expression vector into which a polynucleic acid molecule has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted polynucleic acid molecule will be of an antisense orientation to a target polynucleic acid molecule of interest).

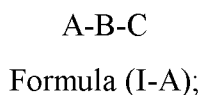
[0103] One embodiment provides an oligonucleotide conjugate of Formula (I) or Formula (I-A):



wherein,

A is a binding moiety;

B is an oligonucleotide comprising a compound of Formula (II) or (IIa).

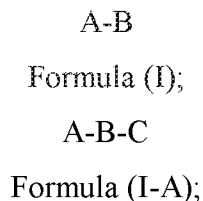


wherein,

A is a binding moiety; and

B is an oligonucleotide comprising a compound of Formula (II) or (IIa)

[0104] C is optionally a polymer. One embodiment provides an oligonucleotide conjugate of Formula (I) or Formula (I-A):

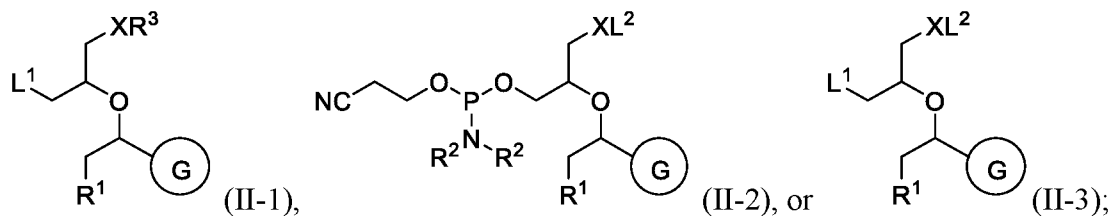


wherein,

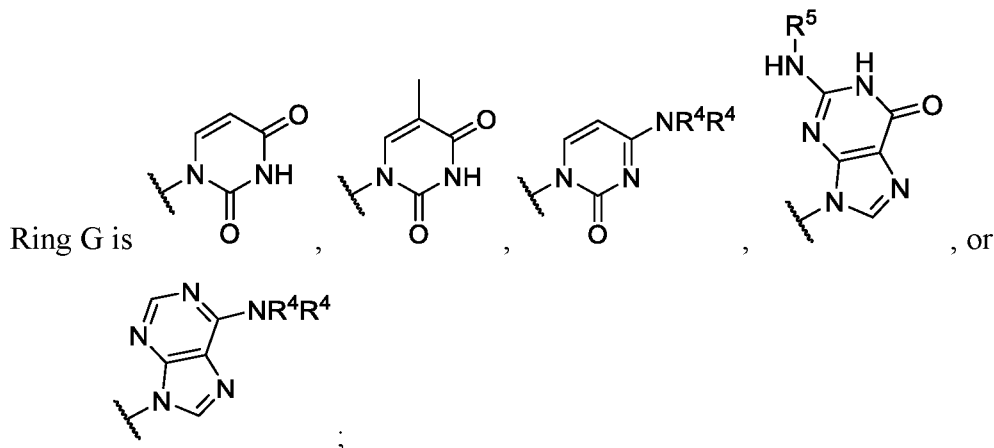
A is a binding moiety;

C is optionally a polymer; and

B is an oligonucleotide comprising a compound of Formula (II-1), (II-2), or (II-3):



wherein,



X is O, NH, or C(=O);

L¹ and L² are each independently absent or a linking moiety selected from a phosphodiester, amide, and ester linkage:

R¹ is hydrogen, deuterium, halogen, substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, substituted or unsubstituted C₁-C₁₀ fluoroalkyl, substituted or unsubstituted C₁-C₂₀ heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted -O-aryl, -OR⁶, -C(=O)R⁶, -C(=O)OR⁶, -C(=O)NR⁷R⁷, -OCH₂C(=O)R⁶, -OCH₂C(=O)OR⁶, or -OCH₂C(=O)NR⁷R⁷;

each R² is independently hydrogen, deuterium, or substituted or unsubstituted C₁-C₁₀ alkyl;

or two R² are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C₃-C₁₀ heterocycloalkyl;

R³ is hydrogen, 4,4'-dimethoxytrityl (DMT), -R⁶, -OR⁶, or -NR⁷R⁷;

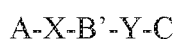
each R⁴ is independently hydrogen, substituted or unsubstituted C₁-C₆alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted benzoyl;

R⁵ is -C(=O)OR⁶;

each R⁶ and R⁷ are independently hydrogen, substituted or unsubstituted C₁-C₆alkyl, substituted or unsubstituted C₁-C₆fluoroalkyl, substituted or unsubstituted C₃-C₆cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

[0105] In some embodiments of the oligonucleotide conjugate of Formula (I), B is a compound having the structure of Formula (II-1). In some embodiments of the oligonucleotide conjugate of Formula (I), B is a compound having the structure of Formula (II-2). In some embodiments of the oligonucleotide conjugate of Formula (I), B is a compound having the structure of Formula (II-3).

[0106] One embodiment provides an oligonucleotide conjugate of Formula (Xa):



Formula (Xa);

wherein,

A is a binding moiety;

B' is an oligonucleotide comprising compound of Formula (II) or (IIa);

C is optionally a polymer;

X is a bond or a first linker; and

Y is a bond or a second linker;

wherein the polynucleotide further comprises one or more additional non-natural nucleotides; and

wherein A and C are not attached to B at the same terminus.

[0107] Another embodiment provides the oligonucleotide comprising a compound of Formula (I) or an oligonucleotide conjugate of Formula (Xa), wherein the oligonucleotide further comprises, at least one modified internucleotide linkage, or at least one inverted abasic moiety.

[0108] Another embodiment provides the oligonucleotide comprising a compound of Formula (I) or an oligonucleotide conjugate of Formula (Xa), wherein the compound of Formula II is located at the 5'-terminus of the oligonucleotide.

[0109] Another embodiment provides the oligonucleotide comprising a compound of Formula (I) or an oligonucleotide conjugate of Formula (Xa), wherein the compound of Formula II is located at an internucleotide linkage of the oligonucleotide.

[0110] Another embodiment provides the oligonucleotide comprising a compound of Formula (I) or an oligonucleotide conjugate of Formula (Xa), wherein the compound of Formula II is further modified at the 2'-position.

[0111] Another embodiment provides the oligonucleotide comprising a compound of Formula (I) or an oligonucleotide conjugate of Formula (Xa), wherein the one or more additional non-

natural nucleotides comprises a 2'-modification selected from 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide.

[0112] Another embodiment provides the oligonucleotide comprising a compound of Formula (I) or an oligonucleotide conjugate of Formula (Xa), wherein the compound of Formula II is selected from locked nucleic acid (LNA) or ethylene nucleic acid (ENA).

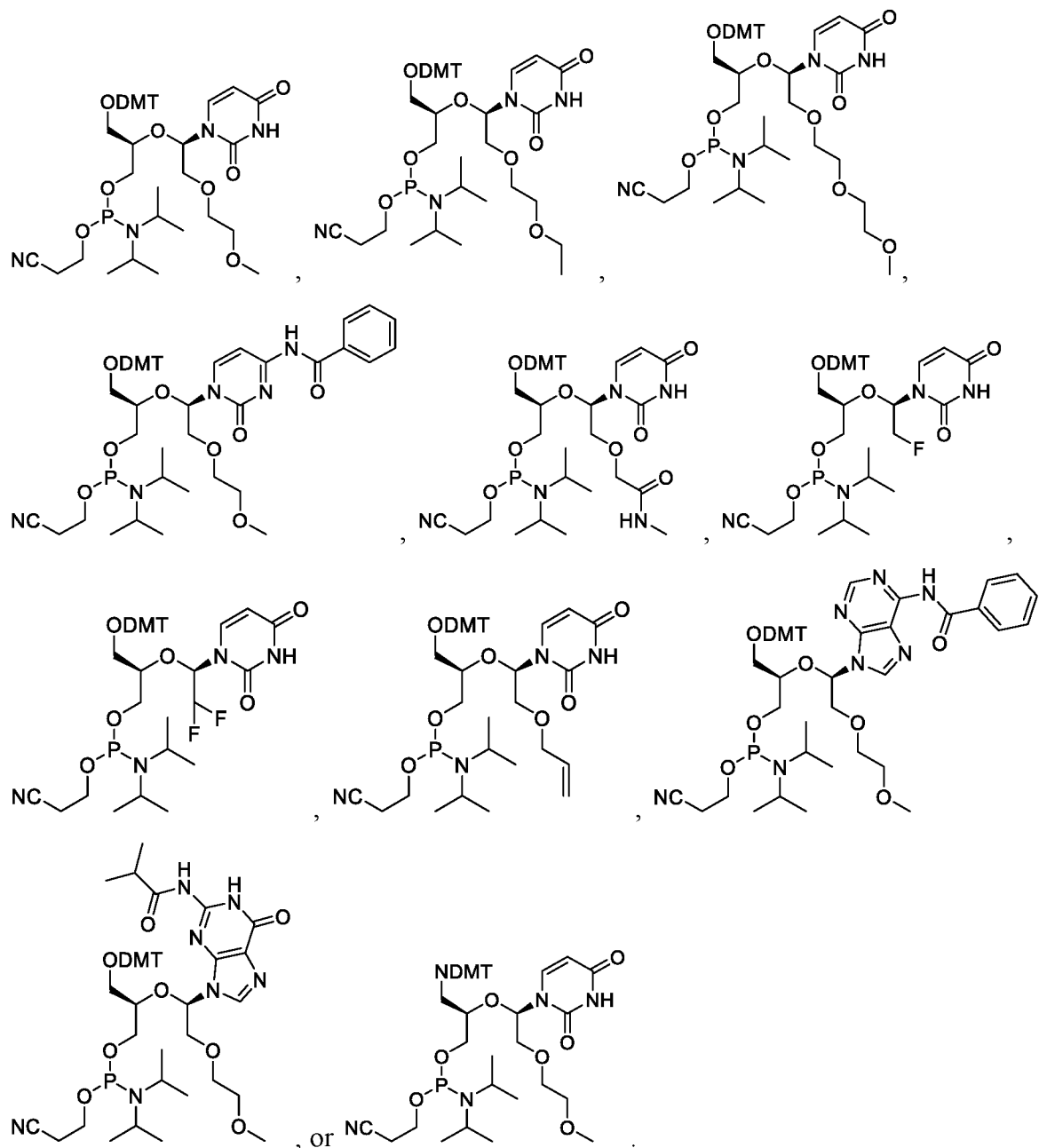
[0113] Another embodiment provides the oligonucleotide comprising a compound of Formula (I) or an oligonucleotide conjugate of Formula (Xa), wherein the at least one inverted abasic moiety is at least one terminus.

[0114] Another embodiment provides the oligonucleotide comprising a compound of Formula (I) or an oligonucleotide conjugate of Formula (Xa), wherein the oligonucleotide is single stranded. Another embodiment provides the oligonucleotide of Formula (Xa), wherein the oligonucleotide is double stranded.

[0115] Another embodiment provides the oligonucleotide comprising a compound of Formula (I) or an oligonucleotide conjugate of Formula (Xa), wherein the oligonucleotide is from 2 to about 100 residues in length. Another embodiment provides the oligonucleotide comprising a compound of Formula (I) or an oligonucleotide conjugate of Formula (Xa), wherein the oligonucleotide is from 2 to about 90 residues in length. Another embodiment provides the oligonucleotide of Formula (I) or (Xa), wherein the oligonucleotide is from 2 to about 80 residues in length. Another embodiment provides the oligonucleotide of Formula (I) or (Xa), wherein the oligonucleotide is from 2 to about 70 residues in length. Another embodiment provides the oligonucleotide of Formula (I) or (Xa), wherein the oligonucleotide is from 2 to about 60 residues in length. Another embodiment provides the oligonucleotide of Formula (I) or (Xa), wherein the oligonucleotide is from 2 to about 50 residues in length. Another embodiment provides the oligonucleotide of Formula (I) or (Xa), wherein the oligonucleotide is from 2 to about 40 residues in length. Another embodiment provides the oligonucleotide of Formula (I) or (Xa), wherein the oligonucleotide is from 2 to about 30 residues in length. Another embodiment provides the oligonucleotide of Formula (I) or (Xa), wherein the oligonucleotide is from 2 to about 20 residues in length. Another embodiment provides the oligonucleotide of Formula (I) or (Xa), wherein the oligonucleotide is from 2 to about 10 residues in length. Another embodiment provides the oligonucleotide of Formula (I) or (Xa), wherein the oligonucleotide is from 8 to about 30 residues in length. Another embodiment provides the oligonucleotide of Formula (Xa), wherein the oligonucleotide is from 10 to about 30 residues in length. Another embodiment

provides the oligonucleotide of Formula (I) or (Xa), wherein the oligonucleotide is from 14 to about 30 residues in length. Another embodiment provides the oligonucleotide of Formula (Xa), wherein the oligonucleotide is from 18 to about 30 residues in length. Another embodiment provides the oligonucleotide of Formula (I) or (Xa), wherein the oligonucleotide is from 22 to about 30 residues in length. Another embodiment provides the oligonucleotide of Formula (I) or (X), wherein the oligonucleotide is from 26 to about 30 residues in length.

[0116] One embodiment provides a compound suitable for the synthesis of oligonucleotides selected from the group:



Conjugation Chemistry

[0117] In some embodiments, a polynucleic acid molecule is conjugated to a binding moiety. In some instances, the binding moiety comprises amino acids, peptides, polypeptides, proteins, antibodies, antigens, toxins, hormones, lipids, nucleotides, nucleosides, sugars, carbohydrates, polymers such as polyethylene glycol and polypropylene glycol, as well as analogs or derivatives of all of these classes of substances. Additional examples of binding moiety also include steroids, such as cholesterol, phospholipids, di-and triacylglycerols, fatty acids, hydrocarbons (e.g., saturated, unsaturated, or contains substitutions), enzyme substrates, biotin, digoxigenin, and polysaccharides. In some instances, the binding moiety is an antibody or binding fragment thereof. In some instances, the polynucleic acid molecule is further conjugated to a polymer, and optionally an endosomolytic moiety.

[0118] In some embodiments, the polynucleic acid molecule is conjugated to the binding moiety by a chemical ligation process. In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a native ligation. In some instances, the conjugation is as described in: Dawson, et al. "Synthesis of proteins by native chemical ligation," *Science* 1994, 266, 776–779; Dawson, et al. "Modulation of Reactivity in Native Chemical Ligation through the Use of Thiol Additives," *J. Am. Chem. Soc.* 1997, 119, 4325–4329; Hackeng, et al. "Protein synthesis by native chemical ligation: Expanded scope by using straightforward methodology," *Proc. Natl. Acad. Sci. USA* 1999, 96, 10068–10073; or Wu, et al. "Building complex glycopeptides: Development of a cysteine-free native chemical ligation protocol," *Angew. Chem. Int. Ed.* 2006, 45, 4116–4125. In some instances, the conjugation is as described in U.S. Patent No. 8,936,910. In some embodiments, the polynucleic acid molecule is conjugated to the binding moiety either site-specifically or non-specifically via native ligation chemistry.

[0119] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing a "traceless" coupling technology (Philochem). In some instances, the "traceless" coupling technology utilizes an N-terminal 1,2-aminothiol group on the binding moiety which is then conjugate with a polynucleic acid molecule containing an aldehyde group. (see Casi *et al.*, "Site-specific traceless coupling of potent cytotoxic drugs to recombinant antibodies for pharmacodelivery," *JACS* **134**(13): 5887-5892 (2012))

[0120] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing an unnatural amino acid incorporated into the binding moiety. In some instances, the unnatural amino acid comprises *p*-acetylphenylalanine (pAcPhe). In some instances, the keto group of pAcPhe is selectively coupled to an alkoxy-amine derivative conjugating moiety to form an oxime bond. (see Axup *et al.*, "Synthesis of site-specific antibody-drug conjugates using unnatural amino acids," *PNAS* **109**(40): 16101-16106 (2012)).

[0121] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing an enzyme-catalyzed process. In some instances, the site-directed method utilizes SMARTag™ technology (Redwood). In some instances, the SMARTag™ technology comprises generation of a formylglycine (FGly) residue from cysteine by formylglycine-generating enzyme (FGE) through an oxidation process under the presence of an aldehyde tag and the subsequent conjugation of FGly to an alkylhydrazine-functionalized polynucleic acid molecule via hydrazino-Pictet-Spengler (HIPS) ligation. (see Wu *et al.*, “Site-specific chemical modification of recombinant proteins produced in mammalian cells by using the genetically encoded aldehyde tag,” *PNAS* **106**(9): 3000-3005 (2009); Agarwal, *et al.*, “A Pictet-Spengler ligation for protein chemical modification,” *PNAS* **110**(1): 46-51 (2013)).

[0122] In some instances, the enzyme-catalyzed process comprises microbial transglutaminase (mTG). In some cases, the polynucleic acid molecule is conjugated to the binding moiety utilizing a microbial transglutaminase catalyzed process. In some instances, mTG catalyzes the formation of a covalent bond between the amide side chain of a glutamine within the recognition sequence and a primary amine of a functionalized polynucleic acid molecule. In some instances, mTG is produced from *Streptomyces mobarensis*. (see Strop *et al.*, “Location matters: site of conjugation modulates stability and pharmacokinetics of antibody drug conjugates,” *Chemistry and Biology* **20**(2) 161-167 (2013))

[0123] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a method as described in PCT Publication No. WO2014/140317, which utilizes a sequence-specific transpeptidase.

[0124] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a method as described in U.S. Patent Publication Nos. 2015/0105539 and 2015/0105540.

Binding Moiety

[0125] In some embodiments, the binding moiety A is a polypeptide. In some instances, the polypeptide is an antibody or its fragment thereof. In some cases, the fragment is a binding fragment. In some instances, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, murine antibody or binding fragment thereof, human antibody or binding fragment thereof, anti-murine antibody (e.g., anti-mouse antibody, anti-rat antibody, etc.), anti-human antibody, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab₂, F(ab')₃ fragments, single-chain variable fragment (scFv), bis-scFv, (scFv)₂, diabody, minibody, nanobody, triabody, tetrabody, disulfide stabilized Fv protein (dsFv), single-domain antibody

(sdAb), Ig NAR, camelid antibody or binding fragment thereof, bispecific antibody or binding fragment thereof, or a chemically modified derivative thereof.

[0126] In some instances, A is an antibody or binding fragment thereof. In some instances, A is a humanized antibody or binding fragment thereof, murine antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab₂, F(ab')₃ fragments, single-chain variable fragment (scFv), bis-scFv, (scFv)₂, diabody, minibody, nanobody, triabody, tetrabody, disulfide stabilized Fv protein ("dsFv"), single-domain antibody (sdAb), Ig NAR, camelid antibody or binding fragment thereof, bispecific antibody or binding fragment thereof, or a chemically modified derivative thereof. In some instances, A is a humanized antibody or binding fragment thereof. In some instances, A is a murine antibody or binding fragment thereof. In some instances, A is a chimeric antibody or binding fragment thereof. In some instances, A is a monoclonal antibody or binding fragment thereof. In some instances, A is a full size antibody. In some instances, A is a monovalent Fab'. In some instances, A is a divalent Fab₂. In some instances, A is a single-chain variable fragment (scFv).

[0127] In some embodiments, the binding moiety A is a bispecific antibody or binding fragment thereof. In some instances, the bispecific antibody is a trifunctional antibody or a bispecific mini-antibody. In some cases, the bispecific antibody is a trifunctional antibody. In some instances, the trifunctional antibody is a full length monoclonal antibody comprising binding sites for two different antigens. Exemplary trifunctional antibodies include catumaxomab (which targets EpCAM and CD3; Fresenius Biotech/Trion Pharma), ertumaxomab (targets HER2/neu/CD3; Fresenius Biotech/Trion Pharma), lymphomun FBT A05 (targets CD20/CD3; Fresenius Biotech/Trion Pharma), RG7221 (RO5520985; targets Angiopoietin 2/VEGF; Roche), RG7597 (targets Her1/Her3; Genentech/Roche), MM141 (targets IGF1R/Her3; Merrimack), ABT122 (targets TNFα/IL17; Abbvie), ABT981 (targets IL1α/IL1β; Abbott), LY3164530 (targets Her1/cMET; Eli Lilly), and TRBS07 (Ektomab; targets GD2/CD3; Trion Research GmbH). Additional exemplary trifunctional antibodies include mAb² from F-star Biotechnology Ltd. In some instances, A is a bispecific trifunctional antibody. In some embodiments, A is a bispecific trifunctional antibody selected from: catumaxomab (which targets EpCAM and CD3; Fresenius Biotech/Trion Pharma), ertumaxomab (targets HER2/neu/CD3; Fresenius Biotech/Trion Pharma), lymphomun FBT A05 (targets CD20/CD3; Fresenius Biotech/Trion Pharma), RG7221 (RO5520985; targets Angiopoietin 2/VEGF; Roche), RG7597 (targets Her1/Her3; Genentech/Roche), MM141 (targets IGF1R/Her3; Merrimack), ABT122 (targets TNFα/IL17; Abbvie), ABT981 (targets IL1α/IL1β; Abbott), LY3164530

(targets Her1/cMET; Eli Lilly), TRBS07 (Ektomab; targets GD2/CD3; Trion Research GmbH), and a mAb² from F-star Biotechnology Ltd.

[0128] In some cases, the bispecific antibody is a bispecific mini-antibody. In some instances, the bispecific mini-antibody comprises divalent Fab₂, F(ab)₃ fragments, bis-scFv, (scFv)₂, diabody, minibody, triabody, tetrabody or a bi-specific T-cell engager (BiTE). In some embodiments, the bi-specific T-cell engager is a fusion protein that contains two single-chain variable fragments (scFvs) in which the two scFvs target epitopes of two different antigens. Exemplary bispecific mini-antibodies include, but are not limited to, DART (dual-affinity re-targeting platform; MacroGenics), blinatumomab (MT103 or AMG103; which targets CD19/CD3; Micromet), MT111 (targets CEA/CD3; Micromet/Amegen), MT112 (BAY2010112; targets PSMA/CD3; Micromet/Bayer), MT110 (AMG 110; targets EPCAM/CD3; Amgen/Micromet), MGD006 (targets CD123/CD3; MacroGenics), MGD007 (targets GPA33/CD3; MacroGenics), BI1034020 (targets two different epitopes on β -amyloid; Ablynx), ALX0761 (targets IL17A/IL17F; Ablynx), TF2 (targets CEA/hepten; Immunomedics), IL-17/IL-34 biAb (BMS), AFM13 (targets CD30/CD16; Affimed), AFM11 (targets CD19/CD3; Affimed), and domain antibodies (dAbs from Domantis/GSK).

[0129] In some embodiments, the binding moiety A is a bispecific mini-antibody. In some instances, A is a bispecific Fab₂. In some instances, A is a bispecific F(ab)₃ fragment. In some cases, A is a bispecific bis-scFv. In some cases, A is a bispecific (scFv)₂. In some embodiments, A is a bispecific diabody. In some embodiments, A is a bispecific minibody. In some embodiments, A is a bispecific triabody. In other embodiments, A is a bispecific tetrabody. In other embodiments, A is a bi-specific T-cell engager (BiTE). In additional embodiments, A is a bispecific mini-antibody selected from: DART (dual-affinity re-targeting platform; MacroGenics), blinatumomab (MT103 or AMG103; which targets CD19/CD3; Micromet), MT111 (targets CEA/CD3; Micromet/Amegen), MT112 (BAY2010112; targets PSMA/CD3; Micromet/Bayer), MT110 (AMG 110; targets EPCAM/CD3; Amgen/Micromet), MGD006 (targets CD123/CD3; MacroGenics), MGD007 (targets GPA33/CD3; MacroGenics), BI1034020 (targets two different epitopes on β -amyloid; Ablynx), ALX0761 (targets IL17A/IL17F; Ablynx), TF2 (targets CEA/hepten; Immunomedics), IL-17/IL-34 biAb (BMS), AFM13 (targets CD30/CD16; Affimed), AFM11 (targets CD19/CD3; Affimed), and domain antibodies (dAbs from Domantis/GSK).

[0130] In some embodiments, the binding moiety A is a trispecific antibody. In some instances, the trispecific antibody comprises F(ab)₃ fragments or a triabody. In some instances, A is a trispecific F(ab)₃ fragment. In some cases, A is a triabody. In some embodiments, A is a trispecific antibody as described in Dimas, *et al.*, "Development of a trispecific antibody

designed to simultaneously and efficiently target three different antigens on tumor cells,” *Mol. Pharmaceutics*, **12**(9): 3490-3501 (2015).

[0131] In some embodiments, the binding moiety A is an antibody or binding fragment thereof that recognizes a cell surface protein. In some instances, the cell surface protein is an antigen expressed by a cancerous cell. Exemplary cancer antigens include, but are not limited to, alpha fetoprotein, ASLG659, B7-H3, BAFF-R, Brevican, CA125 (MUC16), CA15-3, CA19-9, carcinoembryonic antigen (CEA), CA242, CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor), CTLA-4, CXCR5, E16 (LAT1, SLC7A5), FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C), epidermal growth factor, ETBR, Fc receptor-like protein 1 (FCRH1), GEDA, HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen), human chorionic gonadotropin, ICOS, IL-2 receptor, IL20R α , Immunoglobulin superfamily receptor translocation associated 2 (IRTA2), L6, Lewis Y, Lewis X, MAGE-1, MAGE-2, MAGE-3, MAGE 4, MART1, mesothelin, MDP, MPF (SMR, MSLN), MCP1 (CCL2), macrophage inhibitory factor (MIF), MPG, MSG783, mucin, MUC1-KLH, Napi3b (SLC34A2), nectin-4, Neu oncogene product, NCA, placental alkaline phosphatase, prostate specific membrane antigen (PMSA), prostatic acid phosphatase, PSCA hlg, p97, Purinergic receptor P2X ligand-gated ion channel 5 (P2X5), LY64 (Lymphocyte antigen 64 (RP105), gp100, P21, six transmembrane epithelial antigen of prostate (STEAP1), STEAP2, Sema 5b, tumor-associated glycoprotein 72 (TAG-72), transferrin receptor, TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4) and the like. In some instances, the binding moiety is an α -transferrin receptor antibody or binding fragments thereof. In some instances, the binding moiety is an α -human transferrin receptor antibody. In some instances, the binding moiety is an α -human transferrin receptor antibody as described in PCT/US2019/068078, which is incorporated by reference herein.

[0132] In some instances, the cell surface protein comprises clusters of differentiation (CD) cell surface markers. Exemplary CD cell surface markers include, but are not limited to, CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CDw12, CD13, CD14, CD15, CD15s, CD16, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD43, CD44, CD45, CD45RO, CD45RA, CD45RB, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CDw60, CD61, CD62E, CD62L (L-selectin), CD62P, CD63, CD64, CD65, CD66a, CD66b, CD66c, CD66d, CD66e, CD71, CD79 (e.g., CD79a, CD79b), CD90, CD95 (Fas), CD103, CD104, CD125 (IL5RA), CD134 (OX40), CD137

(4-1BB), CD152 (CTLA-4), CD221, CD274, CD279 (PD-1), CD319 (SLAMF7), CD326 (EpCAM), and the like.

[0133] In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes a cancer antigen. In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes alpha fetoprotein, ASLG659, B7-H3, BAFF-R, Brevican, CA125 (MUC16), CA15-3, CA19-9, carcinoembryonic antigen (CEA), CA242, CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor), CTLA-4, CXCR5, E16 (LAT1, SLC7A5), FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C), epidermal growth factor, ETBR, Fc receptor-like protein 1 (FCRH1), GEDA, HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen), human chorionic gonadotropin, ICOS, IL-2 receptor, IL20R α , Immunoglobulin superfamily receptor translocation associated 2 (IRTA2), L6, Lewis Y, Lewis X, MAGE-1, MAGE-2, MAGE-3, MAGE 4, MART1, mesothelin, MCP1 (CCL2), MDP, macrophage inhibitory factor (MIF), MPF (SMR, MSLN), MPG, MSG783, mucin, MUC1-KLH, Napi3b (SLC34A2), nectin-4, Neu oncogene product, NCA, placental alkaline phosphatase, prostate specific membrane antigen (PMSA), prostatic acid phosphatase, PSCA hlg, p97, Purinergic receptor P2X ligand-gated ion channel 5 (P2X5), LY64 (Lymphocyte antigen 64 (RP105), gp100, P21, six transmembrane epithelial antigen of prostate (STEAP1), STEAP2, Sema 5b, tumor-associated glycoprotein 72 (TAG-72), TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4) or a combination thereof.

[0134] In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes a CD cell surface marker. In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CDw12, CD13, CD14, CD15, CD15s, CD16, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD43, CD44, CD45, CD45RO, CD45RA, CD45RB, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CDw60, CD61, CD62E, CD62L (L-selectin), CD62P, CD63, CD64, CD65, CD66a, CD66b, CD66c, CD66d, CD66e, CD71, CD79 (e.g., CD79a, CD79b), CD90, CD95 (Fas), CD103, CD104, CD125 (IL5RA), CD134 (OX40), CD137 (4-1BB), CD152 (CTLA-4), CD221, CD274, CD279 (PD-1), CD319 (SLAMF7), CD326 (EpCAM), or a combination thereof.

[0135] In some embodiments, the antibody or binding fragment thereof comprises zalutumumab (HuMax-EFGr, Genmab), abagovomab (Menarini), abituzumab (Merck),

adecatumumab (MT201), alacizumab pegol, alemtuzumab (Campath®, MabCampath, or Campath-1H; Leukosite), AlloMune (BioTransplant), amatuximab (Morphotek, Inc.), anti-VEGF (Genentech), anatumomab mafenatox, apolizumab (hu1D10), ascrinvacumab (Pfizer Inc.), atezolizumab (MPDL3280A; Genentech/Roche), B43.13 (OvaRex, AltaRex Corporation), basiliximab (Simulect®, Novartis), belimumab (Benlysta®, GlaxoSmithKline), bevacizumab (Avastin®, Genentech), blinatumomab (Blincyto, AMG103; Amgen), BEC2 (ImGlone Systems Inc.), carlumab (Janssen Biotech), catumaxomab (Removab, Trion Pharma), CEAcide (Immunomedics), Cetuximab (Erbix®, ImClone), citatuzumab bogatox (VB6-845), cixutumumab (IMC-A12, ImClone Systems Inc.), conatumumab (AMG 655, Amgen), dacetuzumab (SGN-40, huS2C6; Seattle Genetics, Inc.), daratumumab (Darzalex®, Janssen Biotech), detumomab, drozitumab (Genentech), durvalumab (MedImmune), dusigitumab (MedImmune), edrecolomab (MAb17-1A, Panorex, Glaxo Wellcome), elotuzumab (Empliciti™, Bristol-Myers Squibb), emibetuzumab (Eli Lilly), enavatuzumab (Facet Biotech Corp.), enfortumab vedotin (Seattle Genetics, Inc.), enoblituzumab (MGA271, MacroGenics, Inc.), ensituxumab (Neogenix Oncology, Inc.), epratuzumab (LymphoCide, Immunomedics, Inc.), ertumaxomab (Rexomun®, Trion Pharma), etaracizumab (Abegrin, MedImmune), farletuzumab (MORAb-003, Morphotek, Inc), FBTA05 (Lymphomun, Trion Pharma), ficlatuzumab (AVEO Pharmaceuticals), figitumumab (CP-751871, Pfizer), flanvotumab (ImClone Systems), fresolimumab (GC1008, Aanofti-Aventis), futuximab, glaximab, ganitumab (Amgen), girentuximab (Rencarex®, Willex AG), IMAB362 (Claudiximab, Ganymed Pharmaceuticals AG), imalumab (Baxalta), IMC-1C11 (ImClone Systems), IMC-C225 (Imclone Systems Inc.), imgatuzumab (Genentech/Roche), intetumumab (Centocor, Inc.), ipilimumab (Yervoy®, Bristol-Myers Squibb), iratumumab (Medarex, Inc.), isatuximab (SAR650984, Sanofi-Aventis), labetuzumab (CEA-CIDE, Immunomedics), lexatumumab (ETR2-ST01, Cambridge Antibody Technology), lintuzumab (SGN-33, Seattle Genetics), lucatumumab (Novartis), lumiliximab, mapatumumab (HGS-ETR1, Human Genome Sciences), matuzumab (EMD 72000, Merck), milatuzumab (hLL1, Immunomedics, Inc.), mitumomab (BEC-2, ImClone Systems), narnatumab (ImClone Systems), necitumumab (Portrazza™, Eli Lilly), nesvacumab (Regeneron Pharmaceuticals), nimotuzumab (h-R3, BIOMAb EGFR, TheraCIM, Theraloc, or CIMAherr; Biotech Pharmaceutical Co.), nivolumab (Opdivo®, Bristol-Myers Squibb), obinutuzumab (Gazyva or Gazyvaro; Hoffmann-La Roche), ocaratuzumab (AME-133v, LY2469298; Mentrik Biotech, LLC), ofatumumab (Arzerra®, Genmab), onartuzumab (Genentech), Ontuxizumab (Morphotek, Inc.), oregovomab (OvaRex®, AltaRex Corp.), otlertuzumab (Emergent BioSolutions), panitumumab (ABX-EGF, Amgen), pankomab (Glycotope GMBH), parsatuzumab (Genentech), patritumab, pembrolizumab (Keytruda®,

Merck), pentumomab (Theragyn, Antisoma), pertuzumab (Perjeta, Genentech), pidilizumab (CT-011, Medivation), polatuzumab vedotin (Genentech/Roche), pritumumab, racotumomab (Vaxira®, Recombio), ramucirumab (Cyramza®, ImClone Systems Inc.), rituximab (Rituxan®, Genentech), robatumumab (Schering-Plough), Seribantumab (Sanofi/Merrimack Pharmaceuticals, Inc.), sibrotuzumab, siltuximab (Sylvant™, Janssen Biotech), Smart MI95 (Protein Design Labs, Inc.), Smart ID10 (Protein Design Labs, Inc.), tabalumab (LY2127399, Eli Lilly), taplitumomab paptox, tenatumomab, teprotumumab (Roche), tetulomab, TGN1412 (CD28-SuperMAB or TAB08), tigatuzumab (CD-1008, Daiichi Sankyo), tositumomab, trastuzumab (Herceptin®), tremelimumab (CP-672,206; Pfizer), tucotuzumab celmoleukin (EMD Pharmaceuticals), ublituximab, urelumab (BMS-663513, Bristol-Myers Squibb), volociximab (M200, Biogen Idec), zatuximab, and the like.

[0136] In some embodiments, the binding moiety A comprises zalutumumab (HuMax-EFGr, Genmab), abagovomab (Menarini), abituzumab (Merck), adecatumumab (MT201), alacizumab pegol, alemtuzumab (Campath®, MabCampath, or Campath-1H; Leukosite), AlloMune (BioTransplant), amatuximab (Morphotek, Inc.), anti-VEGF (Genetech), anatumomab mafenatox, apolizumab (hu1D10), ascrinvacumab (Pfizer Inc.), atezolizumab (MPDL3280A; Genentech/Roche), B43.13 (OvaRex, AltaRex Corporation), basiliximab (Simulect®, Novartis), belimumab (Benlysta®, GlaxoSmithKline), bevacizumab (Avastin®, Genentech), blinatumomab (Blinicyto, AMG103; Amgen), BEC2 (ImGlone Systems Inc.), carlumab (Janssen Biotech), catumaxomab (Removab, Trion Pharma), CEAcide (Immunomedics), Cetuximab (Erbix®, ImClone), citatuzumab bogatox (VB6-845), cixutumumab (IMC-A12, ImClone Systems Inc.), conatumumab (AMG 655, Amgen), dacetuzumab (SGN-40, huS2C6; Seattle Genetics, Inc.), daratumumab (Darzalex®, Janssen Biotech), detumomab, drozitumab (Genentech), durvalumab (MedImmune), dusigitumab (MedImmune), edrecolomab (MAb17-1A, Panorex, Glaxo Wellcome), elotuzumab (Empliciti™, Bristol-Myers Squibb), emibetuzumab (Eli Lilly), enavatuzumab (Facet Biotech Corp.), enfortumab vedotin (Seattle Genetics, Inc.), enoblituzumab (MGA271, MacroGenics, Inc.), ensituxumab (Neogenix Oncology, Inc.), epratuzumab (LymphoCide, Immunomedics, Inc.), ertumaxomab (Rexomun®, Trion Pharma), etaracizumab (Abegrin, MedImmune), farletuzumab (MORAb-003, Morphotek, Inc), FBT A05 (Lymphomun, Trion Pharma), ficlatuzumab (AVEO Pharmaceuticals), figitumumab (CP-751871, Pfizer), flanvotumab (ImClone Systems), fresolimumab (GC1008, Aanofi-Aventis), futuximab, glaximab, ganitumab (Amgen), girentuximab (Rencarex®, Willex AG), IMAB362 (Claudiximab, Ganymed Pharmaceuticals AG), imalumab (Baxalta), IMC-1C11 (ImClone Systems), IMC-C225 (Imclone Systems Inc.), imgatuzumab (Genentech/Roche), intetumumab (Centocor, Inc.), ipilimumab (Yervoy®, Bristol-Myers Squibb), iratumumab

(Medarex, Inc.), isatuximab (SAR650984, Sanofi-Aventis), labetuzumab (CEA-CIDE, Immunomedics), lexatumumab (ETR2-ST01, Cambridge Antibody Technology), lintuzumab (SGN-33, Seattle Genetics), lucatumumab (Novartis), lumiliximab, mapatumumab (HGS-ETR1, Human Genome Sciences), matuzumab (EMD 72000, Merck), milatuzumab (hLL1, Immunomedics, Inc.), mitumomab (BEC-2, ImClone Systems), narnatumab (ImClone Systems), necitumumab (Portrazza™, Eli Lilly), nesvacumab (Regeneron Pharmaceuticals), nimotuzumab (h-R3, BIOMAb EGFR, TheraCIM, Theraloc, or CIMAher; Biotech Pharmaceutical Co.), nivolumab (Opdivo®, Bristol-Myers Squibb), obinutuzumab (Gazyva or Gazyvaro; Hoffmann-La Roche), ocaratuzumab (AME-133v, LY2469298; Mentrik Biotech, LLC), ofatumumab (Arzerra®, Genmab), onartuzumab (Genentech), Ontuxizumab (Morphotek, Inc.), oregovomab (OvaRex®, AltaRex Corp.), otlertuzumab (Emergent BioSolutions), panitumumab (ABX-EGF, Amgen), pankomab (Glycotope GMBH), parsatuzumab (Genentech), patritumab, pembrolizumab (Keytruda®, Merck), pentumomab (Theragyn, Antisoma), pertuzumab (Perjeta, Genentech), pidilizumab (CT-011, Medivation), polatuzumab vedotin (Genentech/Roche), pritumumab, racotumomab (Vaxira®, Recombio), ramucirumab (Cyramza®, ImClone Systems Inc.), rituximab (Rituxan®, Genentech), robatumumab (Schering-Plough), Seribantumab (Sanofi/Merrimack Pharmaceuticals, Inc.), sibrotuzumab, siltuximab (Sylvant™, Janssen Biotech), Smart MI95 (Protein Design Labs, Inc.), Smart ID10 (Protein Design Labs, Inc.), tabalumab (LY2127399, Eli Lilly), taplitumomab paptox, tenatumomab, teprotumumab (Roche), tetulomab, TGN1412 (CD28-SuperMAB or TAB08), tigatuzumab (CD-1008, Daiichi Sankyo), tositumomab, trastuzumab (Herceptin®), tremelimumab (CP-672,206; Pfizer), tucotuzumab celmoleukin (EMD Pharmaceuticals), ublituximab, urelumab (BMS-663513, Bristol-Myers Squibb), volociximab (M200, Biogen Idec), or zatuximab. In some embodiments, the binding moiety A is zalutumumab (HuMax-EFGr, by Genmab).

Additional Binding Moieties

[0137] In some embodiments, the binding moiety is a plasma protein. In some instances, the plasma protein comprises albumin. In some instances, the binding moiety A is albumin. In some instances, albumin is conjugated by one or more of a conjugation chemistry described herein to a polynucleic acid molecule. In some instances, albumin is conjugated by native ligation chemistry to a polynucleic acid molecule. In some instances, albumin is conjugated by lysine conjugation to a polynucleic acid molecule.

[0138] In some instances, the binding moiety is a steroid. Exemplary steroids include cholesterol, phospholipids, di-and triacylglycerols, fatty acids, hydrocarbons that are saturated, unsaturated, comprise substitutions, or combinations thereof. In some instances, the steroid is

cholesterol. In some instances, the binding moiety is cholesterol. In some instances, cholesterol is conjugated by one or more of a conjugation chemistry described herein to a polynucleic acid molecule. In some instances, cholesterol is conjugated by native ligation chemistry to a polynucleic acid molecule. In some instances, cholesterol is conjugated by lysine conjugation to a polynucleic acid molecule.

[0139] In some instances, the binding moiety is a polymer, including but not limited to polynucleic acid molecule aptamers that bind to specific surface markers on cells. In this instance the binding moiety is a polynucleic acid that does not hybridize to a target gene or mRNA, but instead is capable of selectively binding to a cell surface marker similarly to an antibody binding to its specific epitope of a cell surface marker.

[0140] In some cases, the binding moiety is a peptide. In some cases, the peptide comprises between about 1 and about 3 kDa. In some cases, the peptide comprises between about 1.2 and about 2.8 kDa, about 1.5 and about 2.5 kDa, or about 1.5 and about 2 kDa. In some instances, the peptide is a bicyclic peptide. In some cases, the bicyclic peptide is a constrained bicyclic peptide. In some instances, the binding moiety is a bicyclic peptide (e.g., bicycles from Bicycle Therapeutics).

In additional cases, the binding moiety is a small molecule. In some instances, the small molecule is an antibody-recruiting small molecule. In some cases, the antibody-recruiting small molecule comprises a target-binding terminus and an antibody-binding terminus, in which the target-binding terminus is capable of recognizing and interacting with a cell surface receptor. For example, in some instances, the target-binding terminus comprising a glutamate urea compound enables interaction with PSMA, thereby, enhances an antibody interaction with a cell (e.g., a cancerous cell) that expresses PSMA. In some instances, a binding moiety is a small molecule described in Zhang et al., "A remote arene-binding site on prostate specific membrane antigen revealed by antibody-recruiting small molecules," J Am Chem Soc. 132(36): 12711-12716 (2010); or McEnaney, et al., "Antibody-recruiting molecules: an emerging paradigm for engaging immune function in treating human disease," ACS Chem Biol. 7(7): 1139-1151 (2012).

Polynucleic Acid Molecule Targets

[0141] In some embodiments, the polynucleic acid molecule B is a polynucleic acid molecule (or polynucleotide) that hybridizes to a target region on an oncogene. In some instances, oncogenes are further classified into several categories: growth factors or mitogens, receptor tyrosine kinases, cytoplasmic tyrosine kinases, cytoplasmic serine/threonine kinases, regulatory GTPases, and transcription factors. Exemplary growth factors include c-Sis. Exemplary

receptor tyrosine kinases include epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), and HER2/neu. Exemplary cytoplasmic tyrosine kinases include Src-family tyrosine kinases, Syk-ZAP-70 family of tyrosine kinases, BTK family of tyrosine kinases, and Abl gene in CML. Exemplary cytoplasmic serine/threonine kinases include Raf kinase and cyclin-dependent kinases. Exemplary regulatory GTPases include Ras family of proteins such as KRAS. Exemplary transcription factors include MYC gene. In some instances, an oncogene described herein comprises an oncogene selected from growth factors or mitogens, receptor tyrosine kinases, cytoplasmic tyrosine kinases, cytoplasmic serine/threonine kinases, regulatory GTPases, or transcription factors. In some embodiments, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of an oncogene selected from growth factors or mitogens, receptor tyrosine kinases, cytoplasmic tyrosine kinases, cytoplasmic serine/threonine kinases, regulatory GTPases, or transcription factors.

[0142] In some embodiments, an oncogene described herein comprises *Abl*, *AKT-2*, *ALK*, *AML1* (or *RUNX1*), *AR*, *AXL*, *BCL-2*, 3, 6, *BRAF*, *c-MYC*, *EGFR*, *ErbB-2* (*Her2*, *Neu*), *Fms*, *FOS*, *GLI1*, *HPRT1*, *IL-3*, *INTS2*, *JUN*, *KIT*, *KS3*, *K-sam*, *LBC* (*AKAP13*), *LCK*, *LMO1*, *LMO2*, *LYL1*, *MAS1*, *MDM2*, *MET*, *MLL* (*KMT2A*), *MOS*, *MYB*, *MYH11/CBFB*, *NOTCH1* (*TAN1*), *NTRK1* (*TRK*), *OST* (*SLC51B*), *PAX5*, *PIM1*, *PRAD-1*, *RAF*, *RAR/PML*, *HRAS*, *KRAS*, *NRAS*, *REL/NGR*, *RET*, *ROS*, *SKI*, *SRC*, *TIAM1*, or *TSC2*. In some embodiments, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of *Abl*, *AKT-2*, *ALK*, *AML1* (or *RUNX1*), *AR*, *AXL*, *BCL-2*, 3, 6, *BRAF*, *c-MYC*, *EGFR*, *ErbB-2* (*Her2*, *Neu*), *Fms*, *FOS*, *GLI1*, *HPRT1*, *IL-3*, *INTS2*, *JUN*, *KIT*, *KS3*, *K-sam*, *LBC* (*AKAP13*), *LCK*, *LMO1*, *LMO2*, *LYL1*, *MAS1*, *MDM2*, *MET*, *MLL* (*KMT2A*), *MOS*, *MYB*, *MYH11/CBFB*, *NOTCH1* (*TAN1*), *NTRK1* (*TRK*), *OST* (*SLC51B*), *PAX5*, *PIM1*, *PRAD-1*, *RAF*, *RAR/PML*, *HRAS*, *KRAS*, *NRAS*, *REL/NGR*, *RET*, *ROS*, *SKI*, *SRC*, *TIAM1*, or *TSC2*.

[0143] In some embodiments, an oncogene described herein comprises *KRAS*, *EGFR*, *AR*, *HPRT1*, *CNNTB1* (β -catenin), or β -catenin associated genes. In some embodiments, the polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *KRAS*, *EGFR*, *AR*, *HPRT1*, *CNNTB1* (β -catenin), or β -catenin associated genes. In some embodiments, the polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *KRAS*. In some embodiments, the polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *EGFR*. In some embodiments, the polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *AR*. In some embodiments, the polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *CNNTB1* (β -catenin). In some embodiments, the

polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *CNNB1* (β -catenin) associated genes. In some instances, the β -catenin associated genes comprise *PIK3CA*, *PIK3CB*, and *Myc*. In some instances, the polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *HPRT1*.

[0144] In some embodiments, the binding moiety A is conjugated according to Formula (I) to a polynucleic acid molecule (B), and optionally a polymer (C), described herein. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 142-255, 256-369, 481-591, 592-702, 3407-14222, 14222-14238, or 14239-14352. In some instances, the polynucleic acid molecule comprises a sequence selected from SEQ ID NOs: 142-255, 256-369, 481-591, 592-702, 3407-14222, 14222-14238, or 14239-14352. In some instances, the polymer C optionally comprises polyalkylene oxide (e.g., polyethylene glycol).

[0145] In some embodiments, the binding moiety A is conjugated to a polynucleic acid molecule (B), and optionally a polymer (C). In some instances, the binding moiety A is an antibody or binding fragment thereof.

[0146] In some embodiments, the binding moiety A is conjugated to a polynucleic acid molecule (B) non-specifically. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a lysine residue or a cysteine residue, in a non-site specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a lysine residue in a non-site specific manner. In some cases, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a cysteine residue in a non-site specific manner. In some instances, the binding moiety A is an antibody or binding fragment thereof.

[0147] In some embodiments, the binding moiety A is conjugated to a polynucleic acid molecule (B) in a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a lysine residue, a cysteine residue, at the 5'-terminus, at the 3'-terminus, an unnatural amino acid, or an enzyme-modified or enzyme-catalyzed residue, via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a lysine residue via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a cysteine residue via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) at the 5'-terminus via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) at the 3'-terminus via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through an unnatural amino acid via a site-specific manner. In

some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through an enzyme-modified or enzyme-catalyzed residue via a site-specific manner. In some instances, the binding moiety A is an antibody or binding fragment thereof.

[0148] In some embodiments, one or more regions of a binding moiety A (e.g., an antibody or binding fragment thereof) is conjugated to a polynucleic acid molecule (B). In some instances, the one or more regions of a binding moiety A comprise the N-terminus, the C-terminus, in the constant region, at the hinge region, or the Fc region of the binding moiety A. In some instances, the polynucleic acid molecule (B) is conjugated to the N-terminus of the binding moiety A (e.g., the N-terminus of an antibody or binding fragment thereof). In some instances, the polynucleic acid molecule (B) is conjugated to the C-terminus of the binding moiety A (e.g., the N-terminus of an antibody or binding fragment thereof). In some instances, the polynucleic acid molecule (B) is conjugated to the constant region of the binding moiety A (e.g., the constant region of an antibody or binding fragment thereof). In some instances, the polynucleic acid molecule (B) is conjugated to the hinge region of the binding moiety A (e.g., the constant region of an antibody or binding fragment thereof). In some instances, the polynucleic acid molecule (B) is conjugated to the Fc region of the binding moiety A (e.g., the constant region of an antibody or binding fragment thereof).

[0149] In some embodiments, one or more polynucleic acid molecule (B) is conjugated to a binding moiety A. In some instances, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or more polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 1 polynucleic acid molecule is conjugated to one binding moiety A. In some instances, about 2 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 3 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 4 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 5 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 6 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 7 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 8 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 9 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 10 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 11 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 12 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 13 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 14 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 15 polynucleic acid molecules are conjugated to one binding moiety A.

In some instances, about 16 polynucleic acid molecules are conjugated to one binding moiety A. In some cases, the one or more polynucleic acid molecules are the same. In other cases, the one or more polynucleic acid molecules are different. In some instances, the binding moiety A is an antibody or binding fragment thereof.

[0150] In some embodiments, the number of polynucleic acid molecule (B) conjugated to a binding moiety A (e.g., an antibody or binding fragment thereof) forms a ratio. In some instances, the ratio is referred to as a DAR (drug-to-antibody) ratio, in which the drug as referred to herein is the polynucleic acid molecule (B). In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 2 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 3 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 4 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 5 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 6 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 7 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 8 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 9 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 10 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 11 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 12 or greater.

[0151] In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A (e.g., an antibody or binding fragment thereof) is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 2. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 3. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 4. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 5. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 6. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 7. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about

8. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 9. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 10. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 11. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 12. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 13. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 14. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 15. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 16.

[0152] In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 1. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 2. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 4. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 6. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 8. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 12.

[0153] In some embodiments, an antibody or its binding fragment is further modified using conventional techniques known in the art, for example, by using amino acid deletion, insertion, substitution, addition, and/or by recombination and/or any other modification (e.g. posttranslational and chemical modifications, such as glycosylation and phosphorylation) known in the art either alone or in combination. In some instances, the modification further comprises a modification for modulating interaction with Fc receptors. In some instances, the one or more modifications include those described in, for example, International Publication No. WO97/34631, which discloses amino acid residues involved in the interaction between the Fc domain and the FcRn receptor. Methods for introducing such modifications in the nucleic acid sequence underlying the amino acid sequence of an antibody or its binding fragment is well known to the person skilled in the art.

[0154] In some instances, an antibody binding fragment further encompasses its derivatives and includes polypeptide sequences containing at least one CDR.

[0155] In some instances, the term “single-chain” as used herein means that the first and second domains of a bi-specific single chain construct are covalently linked, preferably in the form of a co-linear amino acid sequence encodable by a single nucleic acid molecule.

[0156] In some instances, a bispecific single chain antibody construct relates to a construct comprising two antibody derived binding domains. In such embodiments, bi-specific single

chain antibody construct is tandem bi-scFv or diabody. In some instances, a scFv contains a VH and VL domain connected by a linker peptide. In some instances, linkers are of a length and sequence sufficient to ensure that each of the first and second domains can, independently from one another, retain their differential binding specificities.

[0157] In some embodiments, binding to or interacting with as used herein defines a binding/interaction of at least two antigen-interaction-sites with each other. In some instances, antigen-interaction-site defines a motif of a polypeptide that shows the capacity of specific interaction with a specific antigen or a specific group of antigens. In some cases, the binding/interaction is also understood to define a specific recognition. In such cases, specific recognition refers to that the antibody or its binding fragment is capable of specifically interacting with and/or binding to at least two amino acids of each of a target molecule. For example, specific recognition relates to the specificity of the antibody molecule, or to its ability to discriminate between the specific regions of a target molecule. In additional instances, the specific interaction of the antigen-interaction-site with its specific antigen results in an initiation of a signal, e.g. due to the induction of a change of the conformation of the antigen, an oligomerization of the antigen, etc. In further embodiments, the binding is exemplified by the specificity of a "key-lock-principle". Thus in some instances, specific motifs in the amino acid sequence of the antigen-interaction-site and the antigen bind to each other as a result of their primary, secondary or tertiary structure as well as the result of secondary modifications of said structure. In such cases, the specific interaction of the antigen-interaction-site with its specific antigen results as well in a simple binding of the site to the antigen.

[0158] In some instances, specific interaction further refers to a reduced cross-reactivity of the antibody or its binding fragment or a reduced off-target effect. For example, the antibody or its binding fragment that bind to the polypeptide/protein of interest but do not or do not essentially bind to any of the other polypeptides are considered as specific for the polypeptide/protein of interest. Examples for the specific interaction of an antigen-interaction-site with a specific antigen comprise the specificity of a ligand for its receptor, for example, the interaction of an antigenic determinant (epitope) with the antigenic binding site of an antibody.

Production of Antibodies or Binding Fragments Thereof

[0159] In some embodiments, polypeptides described herein (e.g., antibodies and its binding fragments) are produced using any method known in the art to be useful for the synthesis of polypeptides (e.g., antibodies), in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

[0160] In some instances, an antibody or its binding fragment thereof is expressed recombinantly, and the nucleic acid encoding the antibody or its binding fragment is assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., 1994, *BioTechniques* 17:242), which involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0161] Alternatively, a nucleic acid molecule encoding an antibody is optionally generated from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the immunoglobulin) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

[0162] In some instances, an antibody or its binding is optionally generated by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies, e.g., as described by Kohler and Milstein (1975, *Nature* 256:495-497) or, as described by Kozbor et al. (1983, *Immunology Today* 4:72) or Cole et al. (1985 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Alternatively, a clone encoding at least the Fab portion of the antibody is optionally obtained by screening Fab expression libraries (e.g., as described in Huse et al., 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, e.g., Clackson et al., 1991, *Nature* 352:624; Hane et al., 1997 *Proc. Natl. Acad. Sci. USA* 94:4937).

[0163] In some embodiments, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity are used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region, e.g., humanized antibodies.

[0164] In some embodiments, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,694,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54) are adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* are also optionally used (Skerra et al., 1988, *Science* 242:1038-1041).

[0165] In some embodiments, an expression vector comprising the nucleotide sequence of an antibody or the nucleotide sequence of an antibody is transferred to a host cell by conventional techniques (e.g., electroporation, liposomal transfection, and calcium phosphate precipitation), and the transfected cells are then cultured by conventional techniques to produce the antibody. In specific embodiments, the expression of the antibody is regulated by a constitutive, an inducible or a tissue, specific promoter.

[0166] In some embodiments, a variety of host-expression vector systems is utilized to express an antibody or its binding fragment described herein. Such host-expression systems represent vehicles by which the coding sequences of the antibody is produced and subsequently purified, but also represent cells that are, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody or its binding fragment in situ. These include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing an antibody or its binding fragment coding sequences; yeast (e.g., *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing an antibody or its binding fragment coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing an antibody or its binding fragment coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing an antibody or its binding fragment coding sequences; or mammalian cell systems (e.g., COS, CHO, BH, 293, 293T, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g. the adenovirus late promoter; the vaccinia virus 7.5K promoter).

[0167] For long-term, high-yield production of recombinant proteins, stable expression is preferred. In some instances, cell lines that stably express an antibody are optionally engineered. Rather than using expression vectors that contain viral origins of replication, host cells are transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells are then allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn are cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express the antibody or its binding fragments.

[0168] In some instances, a number of selection systems are used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1972, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes are employed in tk⁻, hgp⁻ or ap⁻ cells, respectively. Also, antimetabolite resistance are used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, *Proc. Natl. Acad. Sci. USA* 77:357; O'Hare et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (*Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIB TECH* 11(5):155-215) and hyg^r, which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147).

Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds., 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY.; Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1).

[0169] In some instances, the expression levels of an antibody are increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol. 3. (Academic Press, New York, 1987)). When a marker in the vector system expressing an antibody is amplifiable, an increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the nucleotide sequence of the antibody, production of the antibody will also increase (Crouse et al., 1983, *Mol. Cell Biol.* 3:257).

[0170] In some instances, any method known in the art for purification of an antibody is used, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Polymer Conjugating Moiety

[0171] In some embodiments, a polymer moiety C is further conjugated to a polynucleic acid molecule described herein, a binding moiety described herein, or in combinations thereof. In some instances, a polymer moiety C is conjugated a polynucleic acid molecule. In some cases, a

polymer moiety C is conjugated to a binding moiety. In other cases, a polymer moiety C is conjugated to a polynucleic acid molecule-binding moiety molecule. In additional cases, a polymer moiety C is conjugated, and as discussed under the Therapeutic Molecule Platform section.

[0172] In some instances, the polymer moiety C is a natural or synthetic polymer, consisting of long chains of branched or unbranched monomers, and/or cross-linked network of monomers in two or three dimensions. In some instances, the polymer moiety C includes a polysaccharide, lignin, rubber, or polyalkylene oxide (e.g., polyethylene glycol). In some instances, the at least one polymer moiety C includes, but is not limited to, alpha-, omega-dihydroxypolyethyleneglycol, biodegradable lactone-based polymer, e.g. polyacrylic acid, polylactide acid (PLA), poly(glycolic acid) (PGA), polypropylene, polystyrene, polyolefin, polyamide, polycyanoacrylate, polyimide, polyethyleneterephthalat (PET, PETG), polyethylene terephthalate (PETE), polytetramethylene glycol (PTG), or polyurethane as well as mixtures thereof. As used herein, a mixture refers to the use of different polymers within the same compound as well as in reference to block copolymers. In some cases, block copolymers are polymers wherein at least one section of a polymer is build up from monomers of another polymer. In some instances, the polymer moiety C comprises polyalkylene oxide. In some instances, the polymer moiety C comprises PEG. In some instances, the polymer moiety C comprises polyethylene imide (PEI) or hydroxy ethyl starch (HES).

[0173] In some instances, C is a PEG moiety. In some instances, the PEG moiety is conjugated at the 5' terminus of the polynucleic acid molecule while the binding moiety is conjugated at the 3' terminus of the polynucleic acid molecule. In some instances, the PEG moiety is conjugated at the 3' terminus of the polynucleic acid molecule while the binding moiety is conjugated at the 5' terminus of the polynucleic acid molecule. In some instances, the PEG moiety is conjugated to an internal site of the polynucleic acid molecule. In some instances, the PEG moiety, the binding moiety, or a combination thereof, are conjugated to an internal site of the polynucleic acid molecule. In some instances, the conjugation is a direct conjugation. In some instances, the conjugation is via native ligation.

[0174] In some embodiments, the polyalkylene oxide (e.g., PEG) is a polydispers or monodispers compound. In some instances, polydispers material comprises disperse distribution of different molecular weight of the material, characterized by mean weight (weight average) size and dispersity. In some instances, the monodisperse PEG comprises one size of molecules. In some embodiments, C is poly- or monodispersed polyalkylene oxide (e.g., PEG) and the indicated molecular weight represents an average of the molecular weight of the polyalkylene oxide, e.g., PEG, molecules.

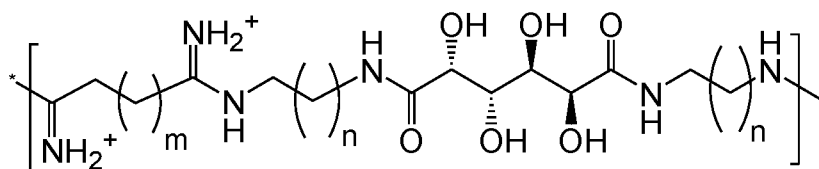
[0175] In some embodiments, the molecular weight of the polyalkylene oxide (e.g., PEG) is about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da.

[0176] In some embodiments, C is polyalkylene oxide (e.g., PEG) and has a molecular weight of about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da. In some embodiments, C is PEG and has a molecular weight of about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da. In some instances, the molecular weight of C is about 200 Da. In some instances, the molecular weight of C is about 300 Da. In some instances, the molecular weight of C is about 400 Da. In some instances, the molecular weight of C is about 500 Da. In some instances, the molecular weight of C is about 600 Da. In some instances, the molecular weight of C is about 700 Da. In some instances, the molecular weight of C is about 800 Da. In some instances, the molecular weight of C is about 900 Da. In some instances, the molecular weight of C is about 1000 Da. In some instances, the molecular weight of C is about 1100 Da. In some instances, the molecular weight of C is about 1200 Da. In some instances, the molecular weight of C is about 1300 Da. In some instances, the molecular weight of C is about 1400 Da. In some instances, the molecular weight of C is about 1450 Da. In some instances, the molecular weight of C is about 1500 Da. In some instances, the molecular weight of C is about 1600 Da. In some instances, the molecular weight of C is about 1700 Da. In some instances, the molecular weight of C is about 1800 Da. In some instances, the molecular weight of C is about 1900 Da. In some instances, the molecular weight of C is about 2000 Da. In some instances, the molecular weight of C is about 2100 Da. In some instances, the molecular weight of C is about 2200 Da. In some instances, the molecular weight of C is about 2300 Da. In some instances, the molecular weight of C is about 2400 Da. In some instances, the molecular weight of C is about 2500 Da. In some instances, the molecular weight of C is about 2600 Da. In some instances, the molecular weight of C is about 2700 Da. In some instances, the molecular weight of C is about 2800 Da. In some instances, the molecular weight of C is about 2900 Da. In some instances, the molecular weight of C is about 3000 Da. In some instances, the molecular weight

of C is about 3250 Da. In some instances, the molecular weight of C is about 3350 Da. In some instances, the molecular weight of C is about 3500 Da. In some instances, the molecular weight of C is about 3750 Da. In some instances, the molecular weight of C is about 4000 Da. In some instances, the molecular weight of C is about 4250 Da. In some instances, the molecular weight of C is about 4500 Da. In some instances, the molecular weight of C is about 4600 Da. In some instances, the molecular weight of C is about 4750 Da. In some instances, the molecular weight of C is about 5000 Da. In some instances, the molecular weight of C is about 5500 Da. In some instances, the molecular weight of C is about 6000 Da. In some instances, the molecular weight of C is about 6500 Da. In some instances, the molecular weight of C is about 7000 Da. In some instances, the molecular weight of C is about 7500 Da. In some instances, the molecular weight of C is about 8000 Da. In some instances, the molecular weight of C is about 10,000 Da. In some instances, the molecular weight of C is about 12,000 Da. In some instances, the molecular weight of C is about 20,000 Da. In some instances, the molecular weight of C is about 35,000 Da. In some instances, the molecular weight of C is about 40,000 Da. In some instances, the molecular weight of C is about 50,000 Da. In some instances, the molecular weight of C is about 60,000 Da. In some instances, the molecular weight of C is about 100,000 Da.

[0177] In some embodiments, the polyalkylene oxide (e.g., PEG) is a discrete PEG, in which the discrete PEG is a polymeric PEG comprising more than one repeating ethylene oxide units. In some instances, a discrete PEG (dPEG) comprises from 2 to 60, from 2 to 50, or from 2 to 48 repeating ethylene oxide units. In some instances, a dPEG comprises about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, 30, 35, 40, 42, 48, 50 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 2 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 3 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 4 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 5 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 6 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 7 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 8 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 9 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 10 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 11 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 12 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 13 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 14 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 15 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 16 or more repeating ethylene oxide units. In some

[0178] In some embodiments, the polymer moiety C comprises a cationic mucic acid-based polymer (cMAP). In some instances, cMPA comprises one or more subunit of at least one repeating subunit, and the subunit structure is represented as Formula (III):



wherein m is independently at each occurrence 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, preferably 4-6 or 5; and n is independently at each occurrence 1, 2, 3, 4, or 5. In some embodiments, m and n are, for example, about 10.

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[0180] In some instances, the polymer moiety C is cMAP-PEG copolymer, an mPEG-cMAP-PEGm triblock polymer, or a cMAP-PEG-cMAP triblock polymer. In some cases, the polymer moiety C is cMAP-PEG copolymer. In other cases, the polymer moiety C is an mPEG-cMAP-PEGm triblock polymer. In additional cases, the polymer moiety C is a cMAP-PEG-cMAP triblock polymer.

Endosomolytic or Cell Membrane Penetration Moiety

[0181] In some embodiments, a molecule of Formula (Xa): A-X₁-B'-X₂-C, further comprises an additional conjugating moiety. In some instances, the additional conjugating moiety is an endosomolytic moiety and/or a cell membrane penetration moiety. In some cases, the endosomolytic moiety is a cellular compartmental release component, such as a compound capable of releasing from any of the cellular compartments known in the art, such as the endosome, lysosome, endoplasmic reticulum (ER), Golgi apparatus, microtubule, peroxisome, or other vesicular bodies with the cell. In some cases, the endosomolytic moiety comprises an endosomolytic polypeptide, an endosomolytic polymer, an endosomolytic lipid, or an endosomolytic small molecule. In some cases, the endosomolytic moiety comprises an endosomolytic polypeptide. In other cases, the endosomolytic moiety comprises an endosomolytic polymer. In some cases, the cell membrane penetration moiety comprises a cell penetrating peptide (CPP). In other cases, the cell membrane penetration moiety comprises a cell penetrating lipid. In other cases, the cell membrane penetration moiety comprises a cell penetrating small molecule.

Endosomolytic and Cell Membrane Penetration Polypeptides

[0182] In some embodiments, a molecule of Formula (Xa): A-X₁-B-X₂-C, is further conjugated with an endosomolytic polypeptide. In some cases, the endosomolytic polypeptide is a pH-dependent membrane active peptide. In some cases, the endosomolytic polypeptide is an amphipathic polypeptide. In additional cases, the endosomolytic polypeptide is a peptidomimetic. In some instances, the endosomolytic polypeptide comprises INF, melittin, meucin, or their respective derivatives thereof. In some instances, the endosomolytic polypeptide comprises INF or its derivatives thereof. In other cases, the endosomolytic polypeptide comprises melittin or its derivatives thereof. In additional cases, the endosomolytic polypeptide comprises meucin or its derivatives thereof. In some instances, the endosomolytic polypeptide comprises Pep-1 (originated from NLS from Simian Virus 40 large antigen and reverse transcriptase of HIV), Pvec (originated from VE-Cadherin), VT5 (originated from synthetic peptide), C105Y (originated from 1-antitrypsin), transportan (originated from Galanin and mastoparan), TP10 (originated from Galanin and mastoparan), MPG (originated from a

hydrophobic domain from the fusion sequence of HIV gp41 and NLS of SV40 T antigen), GH625 (originated from glycoprotein gH of HSV type I), CADY (PPTG1 peptide), GALA (synthetic peptide), INF (Influenza HA2 fusion peptide), HA2E5-TAT (Influenza HA2 subunit of influenza virus X31 strain fusion peptide), HA2-penetratin (Influenza HA2 subunit of influenza virus X31 strain fusion peptide), HA-K4 (Influenza HA2 subunit of influenza virus X31 strain fusion peptide), HA2E4 (Influenza HA2 subunit of influenza virus X31 strain fusion peptide), H5WYG (HA2 analogue), GALA-INF3-(PEG)6-NH (INF3 fusion peptide), or CM18-TAT11 (Cecropin-A-Melittin₂₋₁₂ (CM₁₈) fusion peptide).

[0183] In some cases, the endosomolytic moiety comprises a Bak BH3 polypeptide which induces apoptosis through antagonization of suppressor targets such as Bcl-2 and/or Bcl-x_L. In some instances, the endosomolytic moiety comprises a Bak BH3 polypeptide described in Albarran, *et al.*, "Efficient intracellular delivery of a pro-apoptotic peptide with a pH-responsive carrier," *Reactive & Functional Polymers* **71**: 261-265 (2011).

[0184] In some instances, the endosomolytic moiety comprises a polypeptide (*e.g.*, a cell-penetrating polypeptide) as described in PCT Publication Nos. WO2013/166155 or WO2015/069587.

Endosomolytic Lipids

[0185] In some embodiments, the endosomolytic moiety is a lipid (*e.g.*, a fusogenic lipid). In some embodiments, a molecule of Formula (Xa): A-X₁-B'-X₂-C, is further conjugated with an endosomolytic lipid (*e.g.*, fusogenic lipid). Exemplary fusogenic lipids include 1,2-dioleoyl-sn-3-phosphoethanolamine (DOPE), phosphatidylethanolamine (POPE), palmitoylloleoylphosphatidylcholine (POPC), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-ol (Di-Lin), N-methyl(2,2-di((9Z,12Z)-octadeca-9,12-dienyl)-1,3-dioxolan-4-yl)methanamine (DLin-k-DMA) and N-methyl-2-(2,2-di((9Z,12Z)-octadeca-9,12-dienyl)-1,3-dioxolan-4-yl)ethanamine (XTC). In some instances, an endosomolytic moiety is a lipid (*e.g.*, a fusogenic lipid) described in PCT Publication No. WO09/126,933.

Endosomolytic Small Molecules

[0186] In some embodiments, the endosomolytic moiety is a small molecule. In some embodiments, a molecule of Formula (Xa): A-X₁-B'-X₂-C, is further conjugated with an endosomolytic small molecule. Exemplary small molecules suitable as endosomolytic moieties include, but are not limited to, quinine, chloroquine, hydroxychloroquines, amodiaquins (carnoquines), amopyroquines, primaquines, mefloquines, nivaquines, halofantrines, quinone imines, or a combination thereof. In some instances, quinoline endosomolytic moieties include,

but are not limited to, 7-chloro-4-(4-diethylamino-1-methylbutyl-amino)quinoline (chloroquine); 7-chloro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutyl-amino)quinoline (hydroxychloroquine); 7-fluoro-4-(4-diethylamino-1-methylbutyl-amino)quinoline; 4-(4-diethylamino-1-methylbutylamino) quinoline; 7-hydroxy-4-(4-diethyl-amino-1-methylbutylamino)quinoline; 7-chloro-4-(4-diethylamino-1-butylamino)quinoline (desmethylchloroquine); 7-fluoro-4-(4-diethylamino-1-butylamino)quinoline); 4-(4-diethyl-amino-1-butylamino)quinoline; 7-hydroxy-4-(4-diethylamino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-butylamino)quinoline; 7-fluoro-4-(1-carboxy-4-diethyl-amino-1-butylamino)quinoline; 4-(1-carboxy-4-diethylamino-1-butylamino) quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-fluoro-4-(1-carboxy-4-diethyl-amino-1-methylbutylamino)quinoline; 4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-fluoro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 4-(4-ethyl-(2-hydroxy-ethyl)-amino-1-methylbutylamino-)quinoline; 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; hydroxychloroquine phosphate; 7-chloro-4-(4-ethyl-(2-hydroxyethyl-1)-amino-1-butylamino)quinoline (desmethylhydroxychloroquine); 7-fluoro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino) quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-fluoro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 7-fluoro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 8-[(4-aminopentyl)amino]-6-methoxydihydrochloride quinoline; 1-acetyl-1,2,3,4-tetrahydroquinoline; 8-[(4-aminopentyl)amino]-6-methoxyquinoline dihydrochloride; 1-butyryl-1,2,3,4-tetrahydroquinoline; 3-chloro-4-(4-hydroxy-alpha, alpha'-bis(2-methyl-1-pyrrolidinyl)-2,5-xylidinoquinoline, 4-[(4-diethyl-amino)-1-methylbutyl-amino]-6-methoxyquinoline; 3-fluoro-4-(4-hydroxy-alpha, alpha'-bis(2-methyl-1-pyrrolidinyl)-2,5-xylidinoquinoline, 4-[(4-diethylamino)-1-methylbutyl-amino]-6-methoxyquinoline; 4-(4-hydroxy-alpha, alpha'-bis(2-methyl-1-pyrrolidinyl)-2,5-xylidinoquinoline; 4-[(4-diethylamino)-1-methylbutyl-amino]-6-methoxyquinoline; 3,4-dihydro-1-(2H)-quinolinecarboxyaldehyde; 1,1'-pentamethylene diquinoleinium diiodide; 8-quinolinol sulfate and amino, aldehyde,

carboxylic, hydroxyl, halogen, keto, sulfhydryl and vinyl derivatives or analogs thereof. In some instances, an endosomolytic moiety is a small molecule described in Naisbitt et al (1997, J Pharmacol Exp Therapy 280:884-893) and in U.S. Patent No. 5,736,557.

Cell Penetrating Polypeptide (CPP)

In some embodiments, cell penetrating polypeptide comprises positively charged short peptides with 5–30 amino acids. In some embodiments, cell penetrating polypeptide comprises arginine or lysine rich amino acid sequences. In some embodiments, cell penetrating polypeptide includes any polypeptide or combination thereof, including Antennapedia Penetratin (43–58), HIV-1 TAT protein (48–60), pVEC Cadherin (615–632), Transportan Galanine/Mastoparan, MPG HIV-gp41/SV40 T-antigen, Pep-1 HIV-reverse transcriptase/SV40 T-antigen, Polyarginines, MAP, R6W3, NLS, 8-lysines, ARF (1–22), and Azurin-p28.

Linkers

[0187] In some embodiments, a linker described herein is a cleavable linker or a non-cleavable linker. In some instances, the linker is a cleavable linker. In some instances, the linker is an acid cleavable linker. In some instances, the linker is a non-cleavable linker. In some instances, the linker includes a C₁–C₆ alkyl group (e.g., a C₅, C₄, C₃, C₂, or C₁ alkyl group). In some instances, the linker includes homobifunctional cross linkers, heterobifunctional cross linkers, and the like. In some instances, the linker is a traceless linker (or a zero-length linker). In some instances, the linker is a non-polymeric linker. In some cases, the linker is a non-peptide linker or a linker that does not contain an amino acid residue.

[0188] In some instances, the linker comprises a homobifunctional linker. Exemplary homobifunctional linkers include, but are not limited to, Lomant's reagent dithiobis (succinimidylpropionate) DSP, 3,3'-dithiobis(sulfosuccinimidyl propionate) (DTSSP), disuccinimidyl suberate (DSS), bis(sulfosuccinimidyl)suberate (BS), disuccinimidyl tartrate (DST), disulfosuccinimidyl tartrate (sulfo DST), ethylene glycobis(succinimidylsuccinate) (EGS), disuccinimidyl glutarate (DSG), N,N'-disuccinimidyl carbonate (DSC), dimethyl adipimidate (DMA), dimethyl pimelimidate (DMP), dimethyl suberimidate (DMS), dimethyl-3,3'-dithiobispropionimidate (DTBP), 1,4-di-3'-(2'-pyridyldithio)propionamido)butane (DPDPB), bismaleimidoheptane (BMH), aryl halide-containing compound (DFDNB), such as e.g. 1,5-difluoro-2,4-dinitrobenzene or 1,3-difluoro-4,6-dinitrobenzene, 4,4'-difluoro-3,3'-dinitrophenylsulfone (DFDNPS), bis-[β-(4-azidosalicylamido)ethyl]disulfide (BASED), formaldehyde, glutaraldehyde, 1,4-butanediol diglycidyl ether, adipic acid dihydrazide, carbonyldiimidazole, o-toluidine, 3,3'-dimethylbenzidine, benzidine, α,α'-p-diaminodiphenyl, diiodo-

p-xylene sulfonic acid, N,N'-ethylene-bis(iodoacetamide), or N,N'-hexamethylene-bis(iodoacetamide).

[0189] In some embodiments, the linker comprises a heterobifunctional linker. Exemplary heterobifunctional linker include, but are not limited to, amine-reactive and sulfhydryl cross-linkers such as N-succinimidyl 3-(2-pyridyldithio)propionate (sPDP), long-chain N-succinimidyl 3-(2-pyridyldithio)propionate (LC-sPDP), water-soluble-long-chain N-succinimidyl 3-(2-pyridyldithio) propionate (sulfo-LC-sPDP), succinimidyl-oxycarbonyl- α -methyl- α -(2-pyridyldithio)toluene (sMPT), sulfosuccinimidyl-6-[α -methyl- α -(2-pyridyldithio)toluamido]hexanoate (sulfo-LC-sMPT), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sMCC), sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-sMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBs), m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester (sulfo-MBs), N-succinimidyl(4-iodoacetyl)aminobenzoate (sIAB), sulfosuccinimidyl(4-iodoacetyl)aminobenzoate (sulfo-sIAB), succinimidyl-4-(p-maleimidophenyl)butyrate (sMPB), sulfosuccinimidyl-4-(p-maleimidophenyl)butyrate (sulfo-sMPB), N-(γ -maleimidobutyryloxy)succinimide ester (GMBs), N-(γ -maleimidobutyryloxy)sulfosuccinimide ester (sulfo-GMBs), succinimidyl 6-((iodoacetyl)amino)hexanoate (sIAX), succinimidyl 6-[[6-(((iodoacetyl)amino)hexanoyl)amino]hexanoate (sIAXX), succinimidyl 4-(((iodoacetyl)amino)methyl)cyclohexane-1-carboxylate (sIAC), succinimidyl 6-(((4-iodoacetyl)amino)methyl)cyclohexane-1-carboxylate (sIACX), p-nitrophenyl iodoacetate (NPIA), carbonyl-reactive and sulfhydryl-reactive cross-linkers such as 4-(4-N-maleimidophenyl)butyric acid hydrazide (MPBH), 4-(N-maleimidomethyl)cyclohexane-1-carboxyl-hydrazide-8 (M₂C₂H), 3-(2-pyridyldithio)propionyl hydrazide (PDPH), amine-reactive and photoreactive cross-linkers such as N-hydroxysuccinimidyl-4-azidosalicylic acid (NHs-AsA), N-hydroxysulfosuccinimidyl-4-azidosalicylic acid (sulfo-NHs-AsA), sulfosuccinimidyl-(4-azidosalicylamido)hexanoate (sulfo-NHs-LC-AsA), sulfosuccinimidyl-2-(p-azidosalicylamido)ethyl-1,3'-dithiopropionate (sAsD), N-hydroxysuccinimidyl-4-azidobenzoate (HsAB), N-hydroxysulfosuccinimidyl-4-azidobenzoate (sulfo-HsAB), N-succinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate (sANPAH), sulfosuccinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate (sulfo-sANPAH), N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOs), sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate (sAND), N-succinimidyl-4(4-azidophenyl)1,3'-dithiopropionate (sADP), N-sulfosuccinimidyl(4-azidophenyl)-1,3'-dithiopropionate (sulfo-sADP), sulfosuccinimidyl 4-(p-azidophenyl)butyrate (sulfo-sAPB), sulfosuccinimidyl 2-(7-azido-4-methylcoumarin-3-acetamide)ethyl-1,3'-dithiopropionate (sAED), sulfosuccinimidyl 7-azido-4-methylcoumain-3-acetate (sulfo-

sAMCA), *p*-nitrophenyl diazopyruvate (pNPDP), *p*-nitrophenyl-2-diazo-3,3,3-trifluoropropionate (PNP-DTP), sulfhydryl-reactive and photoreactive cross-linkers such as 1-(*p*-Azidosalicylamido)-4-(iodoacetamido)butane (AsIB), N-[4-(*p*-azidosalicylamido)butyl]-3'-(2'-pyridyldithio)propionamide (APDP), benzophenone-4-iodoacetamide, benzophenone-4-maleimide carbonyl-reactive and photoreactive cross-linkers such as *p*-azidobenzoyl hydrazide (ABH), carboxylate-reactive and photoreactive cross-linkers such as 4-(*p*-azidosalicylamido)butylamine (AsBA), and arginine-reactive and photoreactive cross-linkers such as *p*-azidophenyl glyoxal (APG).

[0190] In some instances, the linker comprises a reactive functional group. In some cases, the reactive functional group comprises a nucleophilic group that is reactive to an electrophilic group present on a binding moiety. Exemplary electrophilic groups include carbonyl groups—such as aldehyde, ketone, carboxylic acid, ester, amide, enone, acyl halide or acid anhydride. In some embodiments, the reactive functional group is aldehyde. Exemplary nucleophilic groups include hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide.

[0191] In some embodiments, the linker comprises a maleimide group. In some instances, the maleimide group is also referred to as a maleimide spacer. In some instances, the maleimide group further encompasses a caproic acid, forming maleimidocaproyl (mc). In some cases, the linker comprises maleimidocaproyl (mc). In some cases, the linker is maleimidocaproyl (mc). In other instances, the maleimide group comprises a maleimidomethyl group, such as succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sMCC) or sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-sMCC) described above.

[0192] In some embodiments, the maleimide group is a self-stabilizing maleimide. In some instances, the self-stabilizing maleimide utilizes diaminopropionic acid (DPR) to incorporate a basic amino group adjacent to the maleimide to provide intramolecular catalysis of tiosuccinimide ring hydrolysis, thereby eliminating maleimide from undergoing an elimination reaction through a retro-Michael reaction. In some instances, the self-stabilizing maleimide is a maleimide group described in Lyon, *et al.*, “Self-hydrolyzing maleimides improve the stability and pharmacological properties of antibody-drug conjugates,” *Nat. Biotechnol.* **32**(10):1059-1062 (2014). In some instances, the linker comprises a self-stabilizing maleimide. In some instances, the linker is a self-stabilizing maleimide.

[0193] In some embodiments, the linker comprises a peptide moiety. In some instances, the peptide moiety comprises at least 2, 3, 4, 5, 6, 7, 8, or more amino acid residues. In some instances, the peptide moiety is a cleavable peptide moiety (e.g., either enzymatically or chemically). In some instances, the peptide moiety is a non-cleavable peptide moiety. In some

instances, the peptide moiety comprises Val-Cit (valine-citrulline), Gly-Gly-Phe-Gly (SEQ ID NO: 14223), Phe-Lys, Val-Lys, Gly-Phe-Lys, Phe-Phe-Lys, Ala-Lys, Val-Arg, Phe-Cit, Phe-Arg, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Ala-Leu-Ala-Leu (SEQ ID NO: 14224), or Gly-Phe-Leu-Gly (SEQ ID NO: 14225). In some instances, the linker comprises a peptide moiety such as: Val-Cit (valine-citrulline), Gly-Gly-Phe-Gly (SEQ ID NO: 14223), Phe-Lys, Val-Lys, Gly-Phe-Lys, Phe-Phe-Lys, Ala-Lys, Val-Arg, Phe-Cit, Phe-Arg, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Ala-Leu-Ala-Leu (SEQ ID NO: 14224), or Gly-Phe-Leu-Gly (SEQ ID NO: 14225). In some cases, the linker comprises Val-Cit. In some cases, the linker is Val-Cit.

[0194] In some embodiments, the linker comprises a benzoic acid group, or its derivatives thereof. In some instances, the benzoic acid group or its derivatives thereof comprise paraaminobenzoic acid (PABA). In some instances, the benzoic acid group or its derivatives thereof comprise gamma-aminobutyric acid (GABA).

[0195] In some embodiments, the linker comprises one or more of a maleimide group, a peptide moiety, and/or a benzoic acid group, in any combination. In some embodiments, the linker comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In some instances, the maleimide group is maleimidocaproyl (mc). In some instances, the peptide group is val-cit. In some instances, the benzoic acid group is PABA. In some instances, the linker comprises a mc-val-cit group. In some cases, the linker comprises a val-cit-PABA group. In additional cases, the linker comprises a mc-val-cit-PABA group.

[0196] In some embodiments, the linker is a self-immolative linker or a self-elimination linker. In some cases, the linker is a self-immolative linker. In other cases, the linker is a self-elimination linker (e.g., a cyclization self-elimination linker). In some instances, the linker comprises a linker described in U.S. Patent No. 9,089,614 or PCT Publication No. WO2015038426.

[0197] In some embodiments, the linker is a dendritic type linker. In some instances, the dendritic type linker comprises a branching, multifunctional linker moiety. In some instances, the dendritic type linker is used to increase the molar ratio of polynucleotide B to the binding moiety A. In some instances, the dendritic type linker comprises PAMAM dendrimers.

[0198] In some embodiments, the linker is a traceless linker or a linker in which after cleavage does not leave behind a linker moiety (e.g., an atom or a linker group) to a binding moiety A, a polynucleotide B, a polymer C, or an endosomolytic moiety D. Exemplary traceless linkers include, but are not limited to, germanium linkers, silicium linkers, sulfur linkers, selenium linkers, nitrogen linkers, phosphorus linkers, boron linkers, chromium linkers, or phenylhydrazide linker. In some cases, the linker is a traceless aryl-triazene linker as described in Hejesen, *et al.*, "A traceless aryl-triazene linker for DNA-directed chemistry," *Org Biomol*

Chem **11**(15): 2493-2497 (2013). In some instances, the linker is a traceless linker described in Blaney, *et al.*, "Traceless solid-phase organic synthesis," *Chem. Rev.* **102**: 2607-2024 (2002). In some instances, a linker is a traceless linker as described in U.S. Patent No. 6,821,783.

[0199] In some instances, the linker comprises a functional group that exerts steric hinderance at the site of bonding between the linker and a conjugating moiety (e.g., A, B, C, or D described herein). In some instances, the steric hinderance is a steric hindrance around a disulfide bond. Exemplary linkers that exhibit steric hinderance comprises a heterobifunctional linker, such as a heterobifunctional linker described above. In some cases, a linker that exhibits steric hinderance comprises SMCC and SPDB.

[0200] In some instances, the linker is an acid cleavable linker. In some instances, the acid cleavable linker comprises a hydrazone linkage, which is susceptible to hydrolytic cleavage. In some cases, the acid cleavable linker comprises a thiomaleamic acid linker. In some cases, the acid cleavable linker is a thiomaleamic acid linker as described in Castaneda, *et al.*, "Acid-cleavable thiomaleamic acid linker for homogeneous antibody-drug conjugation," *Chem. Commun.* **49**: 8187-8189 (2013).

[0201] In some instances, the linker is a linker described in U.S. Patent Nos. 6,884,869; 7,498,298; 8,288,352; 8,609,105; or 8,697,688; U.S. Patent Publication Nos. 2014/0127239; 2013/028919; 2014/286970; 2013/0309256; 2015/037360; or 2014/0294851; or PCT Publication Nos. WO2015057699; WO2014080251; WO2014197854; WO2014145090; or WO2014177042.

[0202] In some embodiments, X, Y, and L are independently a bond or a linker. In some instances, X, Y, and L are independently a bond. In some cases, X, Y, and L are independently a linker.

[0203] In some instances, X is a bond or a linker. In some instances, X is a bond. In some instances, X is a linker. In some instances, the linker is a C₁-C₆ alkyl group. In some cases, X is a C₁-C₆ alkyl group, such as for example, a C₅, C₄, C₃, C₂, or C₁ alkyl group. In some cases, the C₁-C₆ alkyl group is an unsubstituted C₁-C₆ alkyl group. As used in the context of a linker, and in particular in the context of X, alkyl means a saturated straight or branched hydrocarbon radical containing up to six carbon atoms. In some instances, X is a non-polymeric linker. In some instances, X includes a homobifunctional linker or a heterobifunctional linker described *supra*. In some cases, X includes a heterobifunctional linker. In some cases, X includes sMCC. In other instances, X includes a heterobifunctional linker optionally conjugated to a C₁-C₆ alkyl group. In other instances, X includes sMCC optionally conjugated to a C₁-C₆ alkyl group. In additional instances, X does not include a homobifunctional linker or a heterobifunctional linker described *supra*.

[0204] In some instances, Y is a bond or a linker. In some instances, Y is a bond. In other cases, Y is a linker. In some embodiments, Y is a C₁-C₆ alkyl group. In some instances, Y is a homobifunctional linker or a heterobifunctional linker described *supra*. In some instances, Y is a homobifunctional linker described *supra*. In some instances, Y is a heterobifunctional linker described *supra*. In some instances, Y comprises a maleimide group, such as maleimidocaproyl (mc) or a self-stabilizing maleimide group described above. In some instances, Y comprises a peptide moiety, such as Val-Cit. In some instances, Y comprises a benzoic acid group, such as PABA. In additional instances, Y comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In additional instances, Y comprises a mc group. In additional instances, Y comprises a mc-val-cit group. In additional instances, Y comprises a val-cit-PABA group. In additional instances, Y comprises a mc-val-cit-PABA group.

[0205] In some instances, L is a bond or a linker. In some cases, L is a bond. In other cases, L is a linker. In some embodiments, L is a C₁-C₆ alkyl group. In some instances, L is a homobifunctional linker or a heterobifunctional linker described *supra*. In some instances, L is a homobifunctional linker described *supra*. In some instances, L is a heterobifunctional linker described *supra*. In some instances, L comprises a maleimide group, such as maleimidocaproyl (mc) or a self-stabilizing maleimide group described above. In some instances, L comprises a peptide moiety, such as Val-Cit. In some instances, L comprises a benzoic acid group, such as PABA. In additional instances, L comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In additional instances, L comprises a mc group. In additional instances, L comprises a mc-val-cit group. In additional instances, L comprises a val-cit-PABA group. In additional instances, L comprises a mc-val-cit-PABA group.

Methods of Use

[0206] In some embodiments, a composition or a pharmaceutical formulation described herein comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer is used for the treatment of a disease or disorder. In some instances, the disease or disorder is a muscle dystrophy, muscle atrophy, and/or muscle wasting. Muscle dystrophy refers to a loss of muscle mass and/or to a progressive weakening and degeneration of muscles. In some cases, the loss of muscle mass and/or the progressive weakening and degeneration of muscles occurs due to a high rate of protein degradation, a low rate of protein synthesis, or a combination of both. In some cases, a high rate of muscle protein degradation is due to muscle protein catabolism (i.e., the breakdown of muscle protein in order to use amino acids as substrates for gluconeogenesis). In some instances, the disease or disorder is a cancer. In some embodiments, a composition or a

pharmaceutical formulation described herein is used as an immunotherapy for the treatment of a disease or disorder. In some instances, the immunotherapy is an immuno-oncology therapy.

Cancer

[0207] In some embodiments, a composition or a pharmaceutical formulation described herein is used for the treatment of cancer. In some instances, the cancer is a solid tumor. In some instances, the cancer is a hematologic malignancy. In some instances, the cancer is a relapsed or refractory cancer, or a metastatic cancer. In some instances, the solid tumor is a relapsed or refractory solid tumor, or a metastatic solid tumor. In some cases, the hematologic malignancy is a relapsed or refractory hematologic malignancy, or a metastatic hematologic malignancy.

[0208] In some embodiments, the cancer is a solid tumor. In some instances, a composition or a pharmaceutical formulation described herein comprising an oligonucleotide, optionally conjugated to a binding moiety, a polymer, or a combination thereof is used for the treatment of a solid tumor. In some instances, a composition or a pharmaceutical formulation described herein comprising an oligonucleotide, optionally conjugated to a binding moiety, a polymer, or a combination thereof is used for the treatment of anal cancer, appendix cancer, bile duct cancer (i.e., cholangiocarcinoma), bladder cancer, brain tumor, breast cancer, cervical cancer, colon cancer, cancer of Unknown Primary (CUP), esophageal cancer, eye cancer, fallopian tube cancer, gastroenterological cancer, kidney cancer, liver cancer, lung cancer, medulloblastoma, melanoma, oral cancer, ovarian cancer, pancreatic cancer, parathyroid disease, penile cancer, pituitary tumor, prostate cancer, rectal cancer, skin cancer, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, vaginal cancer, or vulvar cancer. In some instances, the solid tumor is a relapsed or refractory solid tumor, or a metastatic solid tumor.

[0209] In some instances, the cancer is a hematologic malignancy. In some instances, a composition or a pharmaceutical formulation described herein comprising an oligonucleotide, optionally conjugated to a binding moiety, a polymer, or a combination thereof is used for the treatment of a hematologic malignancy. In some instances, a composition or a pharmaceutical formulation described herein comprising an oligonucleotide, optionally conjugated to a binding moiety, a polymer, or a combination thereof is used for the treatment of a leukemia, a lymphoma, a myeloma, a non-Hodgkin's lymphoma, or a Hodgkin's lymphoma. In some instances, the hematologic malignancy comprises chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), high risk CLL, a non-CLL/SLL lymphoma, prolymphocytic leukemia (PLL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Waldenström's macroglobulinemia, multiple myeloma, extranodal marginal zone B cell lymphoma, nodal marginal zone B cell lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, primary mediastinal B-cell lymphoma (PMBL),

immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, or lymphomatoid granulomatosis. In some cases, the hematologic malignancy is a relapsed or refractory hematologic malignancy, or a metastatic hematologic malignancy.

[0210] In some instances, the cancer is a KRAS-associated, EGFR-associated, AR-associated cancer, HPRT1-associated cancer, or β -catenin associated cancer. In some instances, a composition or a pharmaceutical formulation described herein comprising an oligonucleotide, optionally conjugated to a binding moiety, a polymer, or a combination thereof is used for the treatment of a KRAS-associated, EGFR-associated, AR-associated cancer, HPRT1-associated cancer, or β -catenin associated cancer. In some instances, a composition or a pharmaceutical formulation described herein comprising an oligonucleotide, optionally conjugated to a binding moiety, a polymer, or a combination thereof is used for the treatment of a KRAS-associated cancer. In some instances, a composition or a pharmaceutical formulation described herein comprising an oligonucleotide, optionally conjugated to a binding moiety, a polymer, or a combination thereof is used for the treatment of an EGFR-associated cancer. In some instances, a composition or a pharmaceutical formulation described herein comprising an oligonucleotide, optionally conjugated to a binding moiety, a polymer, or a combination thereof is used for the treatment of an AR-associated cancer. In some instances, a composition or a pharmaceutical formulation described herein comprising an oligonucleotide, optionally conjugated to a binding moiety, a polymer, or a combination thereof is used for the treatment of an HPRT1-associated cancer. In some instances, a composition or a pharmaceutical formulation described herein comprising an oligonucleotide, optionally conjugated to a binding moiety, a polymer, or a combination thereof is used for the treatment of a β -catenin associated cancer. In some instances, the cancer is a solid tumor. In some instances, the cancer is a hematologic malignancy. In some instances, the solid tumor is a relapsed or refractory solid tumor, or a metastatic solid tumor. In some cases, the hematologic malignancy is a relapsed or refractory hematologic malignancy, or a metastatic hematologic malignancy. In some instances, the cancer comprises bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, glioblastoma multiforme, head and neck cancer, kidney cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, thyroid cancer, acute myeloid leukemia, CLL, DLBCL, or multiple myeloma. In some instances, the β -catenin associated cancer further comprises PIK3C-associated cancer and/or MYC-associated cancer.

Immunotherapy

[0211] In some embodiments, a composition or a pharmaceutical formulation described herein is used as an immunotherapy for the treatment of a disease or disorder. In some instances, the immunotherapy is an immuno-oncology therapy. In some instances, immuno-oncology therapy is categorized into active, passive, or combinatory (active and passive) methods. In active immuno-oncology therapy method, for example, tumor-associated antigens (TAAs) are presented to the immune system to trigger an attack on cancer cells presenting these TAAs. In some instances, the active immune-oncology therapy method includes tumor-targeting and/or immune-targeting agents (e.g., checkpoint inhibitor agents such as monoclonal antibodies), and/or vaccines, such as in situ vaccination and/or cell-based or non-cell based (e.g., dendritic cell-based, tumor cell-based, antigen, anti-idiotypic, DNA, or vector-based) vaccines. In some instances, the cell-based vaccines are vaccines which are generated using activated immune cells obtained from a patient's own immune system which are then activated by the patient's own cancer. In some instances, the active immune-oncology therapy is further subdivided into non-specific active immunotherapy and specific active immunotherapy. In some instances, non-specific active immunotherapy utilizes cytokines and/or other cell signaling components to induce a general immune system response. In some cases, specific active immunotherapy utilizes specific TAAs to elicit an immune response.

[0212] In some embodiments, a composition or a pharmaceutical formulation described herein is used as an active immuno-oncology therapy method for the treatment of a disease or disorder (e.g., cancer). In some embodiments, the composition or a pharmaceutical formulation described herein comprises a tumor-targeting agent. In some instances, the tumor-targeting agent is encompassed by a binding moiety A. In other instances, the tumor-targeting agent is an additional agent used in combination with a molecule of Formula (I). In some instances, the tumor-targeting agent is a tumor-directed polypeptide (e.g., a tumor-directed antibody). In some instances, the tumor-targeting agent is a tumor-directed antibody, which exerts its antitumor activity through mechanisms such as direct killing (e.g., signaling-induced apoptosis), complement-dependent cytotoxicity (CDC), and/or antibody-dependent cell-mediated cytotoxicity (ADCC). In additional instances, the tumor-targeting agent elicits an adaptive immune response, with the induction of antitumor T cells.

[0213] In some embodiments, the binding moiety A is a tumor-directed polypeptide (e.g., a tumor-directed antibody). In some instances, the binding moiety A is a tumor-directed antibody, which exerts its antitumor activity through mechanisms such as direct killing (e.g., signaling-induced apoptosis), complement-dependent cytotoxicity (CDC), and/or antibody-dependent cell-

mediated cytotoxicity (ADCC). In additional instances, the binding moiety A elicits an adaptive immune response, with the induction of antitumor T cells.

[0214] In some embodiments, the composition or a pharmaceutical formulation described herein comprises an immune-targeting agent. In some instances, the immune-targeting agent is encompassed by a binding moiety A. In other instances, the immune-targeting agent is an additional agent used in combination with a molecule of Formula (I). In some instances, the immune-targeting agent comprises cytokines, checkpoint inhibitors, or a combination thereof.

[0215] In some embodiments, the immune-targeting agent is a checkpoint inhibitor. In some cases, an immune checkpoint molecule is a molecule presented on the cell surface of CD4 and/or CD8 T cells. Exemplary immune checkpoint molecules include, but are not limited to, Programmed Death-Ligand 1 (PD-L1, also known as B7-H1, CD274), Programmed Death 1 (PD-1), CTLA-4, B7H1, B7H4, OX-40, CD137, CD40, 2B4, IDO1, IDO2, VISTA, CD27, CD28, PD-L2 (B7-DC, CD273), LAG3, CD80, CD86, PDL2, B7H3, HVEM, BTLA, KIR, GAL9, TIM3, A2aR, MARCO (macrophage receptor with collageneous structure), PS (phosphatidylserine), ICOS (inducible T cell costimulator), HAVCR2, CD276, VTCN1, CD70, and CD160.

[0216] In some instances, an immune checkpoint inhibitor refers to any molecule that modulates or inhibits the activity of an immune checkpoint molecule. In some instances, immune checkpoint inhibitors include antibodies, antibody-derivatives (e.g., Fab fragments, scFvs, minobodies, diabodies), antisense oligonucleotides, siRNA, aptamers, or peptides. In some embodiments, an immune checkpoint inhibitor is an inhibitor of Programmed Death-Ligand 1 (PD-L1, also known as B7-H1, CD274), Programmed Death 1 (PD-1), CTLA-4, PD-L2 (B7-DC, CD273), LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS (inducible T cell costimulator), KIR, LAIR1, LIGHT, MARCO (macrophage receptor with collageneous structure), PS (phosphatidylserine), OX-40, SLAM, TIGHT, VISTA, VTCN1, or any combinations thereof.

[0217] In some embodiments, exemplary checkpoint inhibitors include:

[0218] PD-L1 inhibitors such as Genentech's MPDL3280A (RG7446), Anti-mouse PD-L1 antibody Clone 10F.9G2 (Cat # BE0101) from BioXcell, anti-PD-L1 monoclonal antibody MDX-1105 (BMS-936559) and BMS-935559 from Bristol-Meyer's Squibb, MSB0010718C, mouse anti-PD-L1 Clone 29E.2A3, and AstraZeneca's MEDI4736;

[0219] PD-L2 inhibitors such as GlaxoSmithKline's AMP-224 (Amplimmune), and rHIgM12B7;

[0220] PD-1 inhibitors such as anti-mouse PD-1 antibody Clone J43 (Cat # BE0033-2) from BioXcell, anti-mouse PD-1 antibody Clone RMP1-14 (Cat # BE0146) from BioXcell, mouse anti-PD-1 antibody Clone EH12, Merck's MK-3475 anti-mouse PD-1 antibody (Keytruda, pembrolizumab, lambrolizumab), AnaptysBio's anti-PD-1 antibody known as ANB011, antibody MDX-1 106 (ONO-4538), Bristol-Myers Squibb's human IgG4 monoclonal antibody nivolumab (Opdivo®, BMS-936558, MDX1106), AstraZeneca's AMP-514 and AMP-224, and Pidilizumab (CT-011) from CureTech Ltd;

[0221] CTLA-4 inhibitors such as Bristol Meyers Squibb's anti-CTLA-4 antibody ipilimumab (also known as Yervoy®, MDX-010, BMS-734016 and MDX-101), anti-CTLA4 Antibody, clone 9H10 from Millipore, Pfizer's tremelimumab (CP-675,206, ticilimumab), and anti-CTLA4 antibody clone BNI3 from Abcam;

[0222] LAG3 inhibitors such as anti-Lag-3 antibody clone eBioC9B7W (C9B7W) from eBioscience, anti-Lag3 antibody LS-B2237 from LifeSpan Biosciences, IMP321 (ImmuFact) from Immuteq, anti-Lag3 antibody BMS-986016, and the LAG-3 chimeric antibody A9H12;

[0223] B7-H3 inhibitors such as MGA271;

[0224] KIR inhibitors such as Lirilumab (IPH2101);

[0225] CD137 (41BB) inhibitors such as urelumab (BMS-663513, Bristol-Myers Squibb), PF-05082566 (anti-4-1BB, PF-2566, Pfizer), or XmAb-5592 (Xencor);

[0226] PS inhibitors such as Bavixumab; and inhibitors such as an antibody or fragments (e.g., a monoclonal antibody, a human, humanized, or chimeric antibody) thereof, RNAi molecules, or small molecules to TIM3, CD52, CD30, CD20, CD33, CD27, OX40 (CD134), GITR, ICOS, BTLA (CD272), CD160, 2B4, LAIR1, TIGHT, LIGHT, DR3, CD226, CD2, or SLAM.

[0227] In some embodiments, a binding moiety A comprising an immune checkpoint inhibitor is used for the treatment of a disease or disorder (e.g., cancer). In some instances, the binding moiety A is a bispecific antibody or a binding fragment thereof that comprises an immune checkpoint inhibitor. In some cases, a binding moiety A comprising an inhibitor of Programmed Death-Ligand 1 (PD-L1, also known as B7-H1, CD274), Programmed Death 1 (PD-1), CTLA-4, PD-L2 (B7-DC, CD273), LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS (inducible T cell costimulator), KIR, LAIR1, LIGHT, MARCO (macrophage receptor with collagenous structure), PS (phosphatidylserine), OX-40, SLAM, TIGHT, VISTA, VTCN1, or any combinations thereof, is used for the treatment of a disease or disorder (e.g., cancer).

[0228] In some embodiments, a molecule of Formula (I) in combination with an immune checkpoint inhibitor is used for the treatment of a disease or disorder (e.g., cancer). In some instances, the immune checkpoint inhibitor comprises an inhibitor of Programmed Death-Ligand 1 (PD-L1, also known as B7-H1, CD274), Programmed Death 1 (PD-1), CTLA-4, PD-L2 (B7-DC, CD273), LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS (inducible T cell costimulator), KIR, LAIR1, LIGHT, MARCO (macrophage receptor with collageneous structure), PS (phosphatidylserine), OX-40, SLAM, TIGHT, VISTA, VTCN1, or any combinations thereof. In some cases, a molecule of Formula (I) is used in combination with ipilimumab, tremelimumab, nivolumab, pemrolizumab, pidilizumab, MPDL3280A, MEDI4736, MSB0010718C, MK-3475, or BMS-936559, for the treatment of a disease or disorder (e.g., cancer).

[0229] In some embodiments, the immune-targeting agent is a cytokine. In some cases, cytokine is further subgrouped into chemokine, interferon, interleukin, and tumor necrosis factor. In some embodiments, chemokine plays a role as a chemoattractant to guide the migration of cells, and is classified into four subfamilies: CXC, CC, CX3C, and XC. Exemplary chemokines include chemokines from the CC subfamily: CCL1, CCL2 (MCP-1), CCL3, CCL4, CCL5 (RANTES), CCL6, CCL7, CCL8, CCL9 (or CCL10), CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, and CCL28; the CXC subfamily: CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, and CXCL17; the XC subfamily: XCL1 and XCL2; and the CX3C subfamily CX3CL1.

[0230] Interferon (IFNs) comprises interferon type I (e.g., IFN- α , IFN- β , IFN- ϵ , IFN- κ , and IFN- ω), interferon type II (e.g. IFN- γ), and interferon type III. In some embodiments, IFN- α is further classified into about 13 subtypes which include IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17, and IFNA21.

[0231] Interleukin is expressed by leukocyte or white blood cell and promote the development and differentiation of T and B lymphocytes and hematopoietic cells. Exemplary interleukins include IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8 (CXCL8), IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-35, and IL-36.

[0232] Tumor necrosis factors (TNFs) are a group of cytokines that modulate apoptosis. In some instances, there are about 19 members within the TNF family, including, not limited to, TNF α , lymphotoxin-alpha (LT-alpha), lymphotoxin-beta (LT-beta), T cell antigen gp39

(CD40L), CD27L, CD30L, FASL, 4-1BBL, OX40L, and TNF-related apoptosis inducing ligand (TRAIL).

[0233] In some embodiments, a molecule of Formula (I) in combination with a cytokine is used for the treatment of a disease or disorder (e.g., cancer). In some cases, a molecule of Formula (I) in combination with a chemokine is used for the treatment of a disease or disorder (e.g., cancer). In some cases, a molecule of Formula (I) in combination with an interferon is used for the treatment of a disease or disorder (e.g., cancer). In some cases, a molecule of Formula (I) in combination with an interleukin is used for the treatment of a disease or disorder (e.g., cancer). In some cases, a molecule of Formula (I) in combination with a tumor necrosis factor is used for the treatment of a disease or disorder (e.g., cancer). In some instances, a molecule of Formula (I) in combination with IL-1 β , IL-2, IL-7, IL-8, IL-15, MCP-1 (CCL2), MIP-1 α , RANTES, MCP-3, MIP5, CCL19, CCL21, CXCL2, CXCL9, CXCL10, or CXCL11 is used for the treatment of a disease or disorder (e.g., cancer).

[0234] In some embodiments, the composition or a pharmaceutical formulation described herein comprises a vaccine. In some instances, the vaccine is an in situ vaccination. In some instances, the vaccine is a cell-based vaccine. In some instances, the vaccine is a non-cell based vaccine. In some instances, a molecule of Formula (I) in combination with dendritic cell-based vaccine is used for the treatment of a disease or disorder (e.g., cancer). In some instances, a molecule of Formula (I) in combination with tumor cell-based vaccine is used for the treatment of a disease or disorder (e.g., cancer). In some instances, a molecule of Formula (I) in combination with antigen vaccine is used for the treatment of a disease or disorder (e.g., cancer). In some instances, a molecule of Formula (I) in combination with anti-idiotypic vaccine is used for the treatment of a disease or disorder (e.g., cancer). In some instances, a molecule of Formula (I) in combination with DNA vaccine is used for the treatment of a disease or disorder (e.g., cancer). In some instances, a molecule of Formula (I) in combination with vector-based vaccine is used for the treatment of a disease or disorder (e.g., cancer).

[0235] In some embodiments, a composition or a pharmaceutical formulation described herein is used as a passive immuno-oncology therapy method for the treatment of a disease or disorder (e.g., cancer). The passive method, in some instances, utilizes adoptive immune system components such as T cells, natural killer (NK) T cells, and/or chimeric antigen receptor (CAR) T cells generated exogenously to attack cancer cells.

[0236] In some embodiments, a molecule of Formula (I) in combination with a T-cell based therapeutic agent is used for the treatment of a disease or disorder (e.g., cancer). In some cases, the T-cell based therapeutic agent is an activated T-cell agent that recognizes one or more of a CD cell surface marker described above. In some instances, the T-cell based therapeutic agent

comprises an activated T-cell agent that recognizes one or more of CD2, CD3, CD4, CD5, CD8, CD27, CD28, CD80, CD134, CD137, CD152, CD154, CD160, CD200R, CD223, CD226, CD244, CD258, CD267, CD272, CD274, CD278, CD279, or CD357. In some instances, a molecule of Formula (I) in combination with an activated T-cell agent recognizing one or more of CD2, CD3, CD4, CD5, CD8, CD27, CD28, CD80, CD134, CD137, CD152, CD154, CD160, CD200R, CD223, CD226, CD244, CD258, CD267, CD272, CD274, CD278, CD279, or CD357 is used for the treatment of a disease or disorder (e.g., cancer).

[0237] In some embodiments, a molecule of Formula (I) in combination with natural killer (NK) T cell-based therapeutic agent is used for the treatment of a disease or disorder (e.g., cancer). In some instances, the NK-based therapeutic agent is an activated NK agent that recognizes one or more of a CD cell surface marker described above. In some cases, the NK-based therapeutic agent is an activated NK agent that recognizes one or more of CD2, CD11a, CD11b, CD16, CD56, CD58, CD62L, CD85j, CD158a/b, CD158c, CD158e/f/k, CD158h/j, CD159a, CD162, CD226, CD314, CD335, CD337, CD244, or CD319. In some instances, a molecule of Formula (I) in combination with an activated NK agent recognizing one or more of CD2, CD11a, CD11b, CD16, CD56, CD58, CD62L, CD85j, CD158a/b, CD158c, CD158e/f/k, CD158h/j, CD159a, CD162, CD226, CD314, CD335, CD337, CD244, or CD319 is used for the treatment of a disease or disorder (e.g., cancer).

[0238] In some embodiments, a molecule of Formula (I) in combination with CAR-T cell-based therapeutic agent is used for the treatment of a disease or disorder (e.g., cancer).

[0239] In some embodiments, a molecule of Formula (I) in combination with an additional agent that destabilizes the endosomal membrane (or disrupts the endosomal-lysosomal membrane trafficking) is used for the treatment of a disease or disorder (e.g., cancer). In some instances, the additional agent comprises an antimitotic agent. Exemplary antimitotic agents include, but are not limited to, taxanes such as paclitaxel and docetaxel; vinca alkaloids such as vinblastine, vincristine, vindesine, and vinorelbine; cabazitaxel; colchicine; eribulin; estramustine; etoposide; ixabepilone; podophyllotoxin; teniposide; or griseofulvin. In some instances, the additional agent comprises paclitaxel, docetaxel, vinblastine, vincristine, vindesine, vinorelbine, cabazitaxel, colchicine, eribulin, estramustine, etoposide, ixabepilone, podophyllotoxin, teniposide, or griseofulvin. In some instances, the additional agent comprises taxol. In some instances, the additional agent comprises paclitaxel. In some instances, the additional agent comprises etoposide. In other instances, the additional agent comprises vitamin K3.

[0240] In some embodiments, a composition or a pharmaceutical formulation described herein is used as a combinatory method (including for both active and passive methods) in the treatment of a disease or disorder (e.g., cancer).

Muscle Dystrophy, Muscle Atrophy, Muscle Wasting

[0241] In one embodiment, muscle dystrophy refers to a significant loss in muscle strength. By significant loss in muscle strength is meant a reduction of strength in diseased, injured, or unused muscle tissue in a subject relative to the same muscle tissue in a control subject. In an embodiment, a significant loss in muscle strength is a reduction in strength of at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, or more relative to the same muscle tissue in a control subject. In another embodiment, by significant loss in muscle strength is meant a reduction of strength in unused muscle tissue relative to the muscle strength of the same muscle tissue in the same subject prior to a period of nonuse. In an embodiment, a significant loss in muscle strength is a reduction of at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, or more relative to the muscle strength of the same muscle tissue in the same subject prior to a period of nonuse.

[0242] In another embodiment, muscle dystrophy refers to a significant loss in muscle mass. By significant loss in muscle mass is meant a reduction of muscle volume in diseased, injured, or unused muscle tissue in a subject relative to the same muscle tissue in a control subject. In an embodiment, a significant loss of muscle volume is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, or more relative to the same muscle tissue in a control subject. In another embodiment, by significant loss in muscle mass is meant a reduction of muscle volume in unused muscle tissue relative to the muscle volume of the same muscle tissue in the same subject prior to a period of nonuse. In an embodiment, a significant loss in muscle tissue is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, or more relative to the muscle volume of the same muscle tissue in the same subject prior to a period of nonuse. Muscle volume is optionally measured by evaluating the cross-section area of a muscle such as by Magnetic Resonance Imaging (e.g., by a muscle volume/cross-section area (CSA) MRI method).

[0243] Myotonic dystrophy is a multisystemic neuromuscular disease comprising two main types: myotonic dystrophy type 1 (DM1) and myotonic dystrophy type 2 (DM2). DM1 is caused by a dominantly inherited “CTG” repeat expansion in the gene DM protein kinase (*DMPK*), which when transcribed into mRNA, forms hairpins that bind with high affinity to the

Muscleblind-like (MBNL) family of proteins. MBNL proteins are involved in post-transcriptional splicing and polyadenylation site regulation and loss of the MBNL protein functions lead to downstream accumulation of nuclear foci and increase in mis-splicing events and subsequently to myotonia and other clinical symptoms.

[0244] In some embodiments, described herein is a method of treating muscle dystrophy, muscle atrophy, and/or muscle wasting in a subject, which comprises providing a polynucleic acid molecule described herein or a polynucleic acid molecule conjugate described herein and administering to the subject a therapeutically effective amount of the polynucleic acid molecule or polynucleic acid molecule conjugate to the subject in need thereof to treat the muscular dystrophy muscle atrophy, and/or muscle wasting. In some embodiments, the polynucleic acid molecules or polynucleic acid molecule conjugates target a gene transcripts that are mutated or upregulated such that downregulation, deletion, exon skipping of transcripts are desired to treat the diseases. In some embodiments, the polynucleic acid molecules or polynucleic acid molecule conjugates target DMPK mRNA, DMD mRNA, or GYS1 mRNA.

Pharmaceutical Formulation

[0245] In some embodiments, the pharmaceutical formulations described herein are administered to a subject by multiple administration routes, including but not limited to, parenteral (e.g., intravenous, subcutaneous, intramuscular), oral, intranasal, buccal, rectal, or transdermal administration routes. In some instances, the pharmaceutical composition described herein is formulated for parenteral (e.g., intravenous, subcutaneous, intramuscular) administration. In other instances, the pharmaceutical composition described herein is formulated for oral administration. In still other instances, the pharmaceutical composition described herein is formulated for intranasal administration.

[0246] In some embodiments, the pharmaceutical formulations include, but are not limited to, aqueous liquid dispersions, self-emulsifying dispersions, solid solutions, liposomal dispersions, aerosols, solid dosage forms, powders, immediate-release formulations, controlled-release formulations, fast melt formulations, tablets, capsules, pills, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations (e.g., nanoparticle formulations), and mixed immediate and controlled release formulations.

[0247] In some instances, the pharmaceutical formulation includes multiparticulate formulations. In some instances, the pharmaceutical formulation includes nanoparticle formulations. In some instances, nanoparticles comprise cMAP, cyclodextrin, or lipids. In some cases, nanoparticles comprise solid lipid nanoparticles, polymeric nanoparticles, self-emulsifying nanoparticles, liposomes, microemulsions, or micellar solutions. Additional

exemplary nanoparticles include, but are not limited to, paramagnetic nanoparticles, superparamagnetic nanoparticles, metal nanoparticles, fullerene-like materials, inorganic nanotubes, dendrimers (such as with covalently attached metal chelates), nanofibers, nanohorns, nano-onions, nanorods, nanoropes and quantum dots. In some instances, a nanoparticle is a metal nanoparticle, e.g., a nanoparticle of scandium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, yttrium, zirconium, niobium, molybdenum, ruthenium, rhodium, palladium, silver, cadmium, hafnium, tantalum, tungsten, rhenium, osmium, iridium, platinum, gold, gadolinium, aluminum, gallium, indium, tin, thallium, lead, bismuth, magnesium, calcium, strontium, barium, lithium, sodium, potassium, boron, silicon, phosphorus, germanium, arsenic, antimony, and combinations, alloys or oxides thereof.

[0248] In some instances, a nanoparticle includes a core or a core and a shell, as in a core-shell nanoparticle.

[0249] In some instances, a nanoparticle is further coated with molecules for attachment of functional elements (e.g., with one or more of a polynucleic acid molecule or binding moiety described herein). In some instances, a coating comprises chondroitin sulfate, dextran sulfate, carboxymethyl dextran, alginic acid, pectin, carrageenan, fucoidan, agarose, porphyrin, karaya gum, gellan gum, xanthan gum, hyaluronic acids, glucosamine, galactosamine, chitin (or chitosan), polyglutamic acid, polyaspartic acid, lysozyme, cytochrome C, ribonuclease, trypsinogen, chymotrypsinogen, α -chymotrypsin, polylysine, polyarginine, histone, protamine, ovalbumin, dextrin, or cyclodextrin. In some instances, a nanoparticle comprises a graphene-coated nanoparticle.

[0250] In some cases, a nanoparticle has at least one dimension of less than about 500nm, 400nm, 300nm, 200nm, or 100nm.

[0251] In some instances, the nanoparticle formulation comprises paramagnetic nanoparticles, superparamagnetic nanoparticles, metal nanoparticles, fullerene-like materials, inorganic nanotubes, dendrimers (such as with covalently attached metal chelates), nanofibers, nanohorns, nano-onions, nanorods, nanoropes or quantum dots. In some instances, a polynucleic acid molecule or a binding moiety described herein is conjugated either directly or indirectly to the nanoparticle. In some instances, at least 1, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more polynucleic acid molecules or binding moieties described herein are conjugated either directly or indirectly to a nanoparticle.

[0252] In some embodiments, the pharmaceutical formulations include a carrier or carrier materials selected on the basis of compatibility with the composition disclosed herein, and the release profile properties of the desired dosage form. Exemplary carrier materials include, e.g., binders, suspending agents, disintegration agents, filling agents, surfactants, solubilizers,

stabilizers, lubricants, wetting agents, diluents, and the like. Pharmaceutically compatible carrier materials include, but are not limited to, acacia, gelatin, colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, polyvinylpyrrolidone (PVP), cholesterol, cholesterol esters, sodium caseinate, soy lecithin, taurocholic acid, phosphatidylcholine, sodium chloride, tricalcium phosphate, dipotassium phosphate, cellulose and cellulose conjugates, sugars sodium stearyl lactylate, carrageenan, monoglyceride, diglyceride, pregelatinized starch, and the like. See, e.g., *Remington: The Science and Practice of Pharmacy*, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, N.Y., 1980; and *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Seventh Ed. (Lippincott Williams & Wilkins 1999).

[0253] In some instances, the pharmaceutical formulations further include pH-adjusting agents or buffering agents which include acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids; bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate and tris-hydroxymethylaminomethane; and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases and buffers are included in an amount required to maintain pH of the composition in an acceptable range.

[0254] In some instances, the pharmaceutical formulation includes one or more salts in an amount required to bring osmolality of the composition into an acceptable range. Such salts include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions; suitable salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite and ammonium sulfate.

[0255] In some instances, the pharmaceutical formulations further include diluent which are used to stabilize compounds because they can provide a more stable environment. Salts dissolved in buffered solutions (which also can provide pH control or maintenance) are utilized as diluents in the art, including, but not limited to a phosphate buffered saline solution. In certain instances, diluents increase bulk of the composition to facilitate compression or create sufficient bulk for homogenous blend for capsule filling. Such compounds can include e.g., lactose, starch, mannitol, sorbitol, dextrose, microcrystalline cellulose such as Avicel[®]; dibasic calcium phosphate, dicalcium phosphate dihydrate; tricalcium phosphate, calcium phosphate; anhydrous lactose, spray-dried lactose; pregelatinized starch, compressible sugar, such as Di-Pac[®] (Amstar); mannitol, hydroxypropylmethylcellulose, hydroxypropylmethylcellulose acetate stearate, sucrose-based diluents, confectioner's sugar; monobasic calcium sulfate monohydrate,

calcium sulfate dihydrate; calcium lactate trihydrate, dextrates; hydrolyzed cereal solids, amylose; powdered cellulose, calcium carbonate; glycine, kaolin; mannitol, sodium chloride; inositol, bentonite, and the like.

[0256] In some cases, the pharmaceutical formulations include disintegration agents or disintegrants to facilitate the breakup or disintegration of a substance. The term “disintegrate” include both the dissolution and dispersion of the dosage form when contacted with gastrointestinal fluid. Examples of disintegration agents include a starch, e.g., a natural starch such as corn starch or potato starch, a pregelatinized starch such as National 1551 or Amijel[®], or sodium starch glycolate such as Promogel[®] or Explotab[®], a cellulose such as a wood product, methylcrystalline cellulose, e.g., Avicel[®], Avicel[®] PH101, Avicel[®] PH102, Avicel[®] PH105, Elcema[®] P100, Emcocel[®], Vivacel[®], Ming Tia[®], and Solka-Floc[®], methylcellulose, croscarmellose, or a cross-linked cellulose, such as cross-linked sodium carboxymethylcellulose (Ac-Di-Sol[®]), cross-linked carboxymethylcellulose, or cross-linked croscarmellose, a cross-linked starch such as sodium starch glycolate, a cross-linked polymer such as crospovidone, a cross-linked polyvinylpyrrolidone, alginate such as alginic acid or a salt of alginic acid such as sodium alginate, a clay such as Veegum[®] HV (magnesium aluminum silicate), a gum such as agar, guar, locust bean, Karaya, pectin, or tragacanth, sodium starch glycolate, bentonite, a natural sponge, a surfactant, a resin such as a cation-exchange resin, citrus pulp, sodium lauryl sulfate, sodium lauryl sulfate in combination starch, and the like.

[0257] In some instances, the pharmaceutical formulations include filling agents such as lactose, calcium carbonate, calcium phosphate, dibasic calcium phosphate, calcium sulfate, microcrystalline cellulose, cellulose powder, dextrose, dextrates, dextran, starches, pregelatinized starch, sucrose, xylitol, lactitol, mannitol, sorbitol, sodium chloride, polyethylene glycol, and the like.

[0258] Lubricants and glidants are also optionally included in the pharmaceutical formulations described herein for preventing, reducing or inhibiting adhesion or friction of materials. Exemplary lubricants include, e.g., stearic acid, calcium hydroxide, talc, sodium stearyl fumerate, a hydrocarbon such as mineral oil, or hydrogenated vegetable oil such as hydrogenated soybean oil (Sterotex[®]), higher fatty acids and their alkali-metal and alkaline earth metal salts, such as aluminum, calcium, magnesium, zinc, stearic acid, sodium stearates, glycerol, talc, waxes, Stearowet[®], boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine, a polyethylene glycol (e.g., PEG-4000) or a methoxypolyethylene glycol such as Carbowax[™], sodium oleate, sodium benzoate, glyceryl behenate, polyethylene glycol, magnesium or sodium lauryl sulfate, colloidal silica such as Syloid[™], Cab-O-Sil[®], a starch such as corn starch, silicone oil, a surfactant, and the like.

[0259] Plasticizers include compounds used to soften the microencapsulation material or film coatings to make them less brittle. Suitable plasticizers include, e.g., polyethylene glycols such as PEG 300, PEG 400, PEG 600, PEG 1450, PEG 3350, and PEG 800, stearic acid, propylene glycol, oleic acid, triethyl cellulose and triacetin. Plasticizers can also function as dispersing agents or wetting agents.

[0260] Solubilizers include compounds such as triacetin, triethylcitrate, ethyl oleate, ethyl caprylate, sodium lauryl sulfate, sodium docusate, vitamin E TPGS, dimethylacetamide, N-methylpyrrolidone, N-hydroxyethylpyrrolidone, polyvinylpyrrolidone, hydroxypropylmethyl cellulose, hydroxypropyl cyclodextrins, ethanol, n-butanol, isopropyl alcohol, cholesterol, bile salts, polyethylene glycol 200-600, glycofurol, transcitol, propylene glycol, dimethyl isosorbide, and the like.

[0261] Stabilizers include compounds such as any antioxidation agents, buffers, acids, preservatives and the like.

[0262] Suspending agents include compounds such as polyvinylpyrrolidone, e.g., polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, vinyl pyrrolidone/vinyl acetate copolymer (S630), polyethylene glycol, e.g., the polyethylene glycol can have a molecular weight of about 300 to about 6000, or about 3350 to about 4000, or about 7000 to about 5400, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, hydroxymethylcellulose acetate stearate, polysorbate-80, hydroxyethylcellulose, sodium alginate, gums, such as, e.g., gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulosics, such as, e.g., sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone and the like.

[0263] Surfactants include compounds such as sodium lauryl sulfate, sodium docusate, Tween 60 or 80, triacetin, vitamin E TPGS, sorbitan monooleate, polyoxyethylene sorbitan monooleate, polysorbates, polaxomers, bile salts, glyceryl monostearate, copolymers of ethylene oxide and propylene oxide, e.g., Pluronic[®] (BASF), and the like. Additional surfactants include polyoxyethylene fatty acid glycerides and vegetable oils, e.g., polyoxyethylene (60) hydrogenated castor oil; and polyoxyethylene alkylethers and alkylphenyl ethers, e.g., octoxynol 10, octoxynol 40. Sometimes, surfactants is included to enhance physical stability or for other purposes.

[0264] Viscosity enhancing agents include, e.g., methyl cellulose, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose,

hydroxypropylmethyl cellulose acetate stearate, hydroxypropylmethyl cellulose phthalate, carbomer, polyvinyl alcohol, alginates, acacia, chitosans and combinations thereof.

[0265] Wetting agents include compounds such as oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, sodium docusate, sodium oleate, sodium lauryl sulfate, sodium doccusate, triacetin, Tween 80, vitamin E TPGS, ammonium salts and the like.

Therapeutic Regimens

[0266] In some embodiments, the pharmaceutical compositions described herein are administered for therapeutic applications. In some embodiments, the pharmaceutical composition is administered once per day, twice per day, three times per day or more. The pharmaceutical composition is administered daily, every day, every alternate day, five days a week, once a week, every other week, two weeks per month, three weeks per month, once a month, twice a month, three times per month, or more. The pharmaceutical composition is administered for at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 18 months, 2 years, 3 years, or more.

[0267] In some embodiments, one or more pharmaceutical compositions are administered simultaneously, sequentially, or at an interval period of time. In some embodiments, one or more pharmaceutical compositions are administered simultaneously. In some cases, one or more pharmaceutical compositions are administered sequentially. In additional cases, one or more pharmaceutical compositions are administered at an interval period of time (e.g., the first administration of a first pharmaceutical composition is on day one followed by an interval of at least 1, 2, 3, 4, 5, or more days prior to the administration of at least a second pharmaceutical composition).

[0268] In some embodiments, two or more different pharmaceutical compositions are coadministered. In some instances, the two or more different pharmaceutical compositions are coadministered simultaneously. In some cases, the two or more different pharmaceutical compositions are coadministered sequentially without a gap of time between administrations. In other cases, the two or more different pharmaceutical compositions are coadministered sequentially with a gap of about 0.5 hour, 1 hour, 2 hour, 3 hour, 12 hours, 1 day, 2 days, or more between administrations.

[0269] In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the composition is given continuously; alternatively, the dose of the composition being administered is temporarily reduced or temporarily suspended for a certain

length of time (i.e., a “drug holiday”). In some instances, the length of the drug holiday varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, or 365 days. The dose reduction during a drug holiday is from 10%-100%, including, by way of example only, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

[0270] Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, are optionally reduced, as a function of the symptoms, to a level at which the improved disease, disorder or condition is retained.

[0271] In some embodiments, the amount of a given agent that correspond to such an amount varies depending upon factors such as the particular compound, the severity of the disease, the identity (e.g., weight) of the subject or host in need of treatment, but nevertheless is routinely determined in a manner known in the art according to the particular circumstances surrounding the case, including, e.g., the specific agent being administered, the route of administration, and the subject or host being treated. In some instances, the desired dose is conveniently presented in a single dose or as divided doses administered simultaneously (or over a short period of time) or at appropriate intervals, for example as two, three, four or more sub-doses per day.

[0272] The foregoing ranges are merely suggestive, as the number of variables in regard to an individual treatment regime is large, and considerable excursions from these recommended values are not uncommon. Such dosages are altered depending on a number of variables, not limited to the activity of the compound used, the disease or condition to be treated, the mode of administration, the requirements of the individual subject, the severity of the disease or condition being treated, and the judgment of the practitioner.

[0273] In some embodiments, toxicity and therapeutic efficacy of such therapeutic regimens are determined by standard pharmaceutical procedures in cell cultures or experimental animals, including, but not limited to, the determination of the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index and it is expressed as the ratio between LD₅₀ and ED₅₀. Compounds exhibiting high therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with minimal toxicity. The dosage

varies within this range depending upon the dosage form employed and the route of administration utilized.

Kits/Article of Manufacture

[0274] Disclosed herein, in certain embodiments, are kits and articles of manufacture for use with one or more of the compositions and methods described herein. Such kits include a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. In one embodiment, the containers are formed from a variety of materials such as glass or plastic.

[0275] The articles of manufacture provided herein contain packaging materials. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, bags, containers, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment.

[0276] For example, the container(s) include a molecule of Formula (Xa): A-X-B'-Y-C, as disclosed herein. Such kits optionally include an identifying description or label or instructions relating to its use in the methods described herein.

[0277] A kit typically includes labels listing contents and/or instructions for use and package inserts with instructions for use. A set of instructions will also typically be included.

[0278] In one embodiment, a label is on or associated with the container. In one embodiment, a label is on a container when letters, numbers, or other characters forming the label are attached, molded or etched into the container itself; a label is associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. In one embodiment, a label is used to indicate that the contents are to be used for a specific therapeutic application. The label also indicates directions for use of the contents, such as in the methods described herein.

[0279] In certain embodiments, the pharmaceutical compositions are presented in a pack or dispenser device which contains one or more unit dosage forms containing a compound provided herein. The pack, for example, contains metal or plastic foil, such as a blister pack. In one embodiment, the pack or dispenser device is accompanied by instructions for administration. In one embodiment, the pack or dispenser is also accompanied with a notice associated with the container in form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the drug for human or veterinary administration. Such notice, for example,

is the labeling approved by the U.S. Food and Drug Administration for prescription drugs, or the approved product insert. In one embodiment, compositions containing a compound provided herein formulated in a compatible pharmaceutical carrier are also prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Certain Terminology

[0280] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the claimed subject matter belongs. It is to be understood that the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of any subject matter claimed. In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise. In this application, the use of “or” means “and/or” unless stated otherwise. Furthermore, use of the term “including” as well as other forms, such as “include”, “includes,” and “included,” is not limiting.

[0281] As used herein, ranges and amounts can be expressed as “about” a particular value or range. About also includes the exact amount. Hence “about 5 μ L” means “about 5 μ L” and also “5 μ L.” Generally, the term “about” includes an amount that is expected to be within experimental error.

[0282] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0283] As used herein, the terms “individual(s)”, “subject(s)” and “patient(s)” mean any mammal. In some embodiments, the mammal is a human. In some embodiments, the mammal is a non-human. None of the terms require or are limited to situations characterized by the supervision (e.g. constant or intermittent) of a health care worker (e.g. a doctor, a registered nurse, a nurse practitioner, a physician’s assistant, an orderly or a hospice worker).

Chemical Definitions

[0284] The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical arts.

[0285] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., $-\text{CH}_2\text{O}-$ is equivalent to $-\text{OCH}_2-$.

[0286] The term “alkyl,” by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched carbon chain (or carbon), or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include mono-, di- and multivalent radicals, having the number of carbon atoms designated (i.e., C₁-C₁₀ means one to ten carbons). Alkyl is an uncyclized chain. Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, (cyclohexyl)methyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. An alkoxy is an alkyl attached to the remainder of the molecule via an oxygen linker (-O-).

[0287] The term “alkylene,” by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkyl, as exemplified, but not limited by, -CH₂CH₂CH₂CH₂-. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred herein. A “lower alkyl” or “lower alkylene” is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms. The term “alkenylene,” by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkene.

[0288] The term “heteroalkyl,” by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or combinations thereof, including at least one carbon atom and at least one heteroatom (e.g., O, N, P, Si, and S), and wherein the nitrogen and sulfur atoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) (e.g., N, S, Si, or P) may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Heteroalkyl is an uncyclized chain. Examples include, but are not limited to: -CH₂-CH₂-O-CH₃, -CH₂-CH₂-NH-CH₃, -CH₂-CH₂-N(CH₃)-CH₃, -CH₂-S-CH₂-CH₃, -CH₂-CH₂-, -S(O)-CH₃, -CH₂-CH₂-S(O)₂-CH₃, -CH=CH-O-CH₃, -Si(CH₃)₃-, -CH₂-CH=N-OCH₃, -CH=CH-N(CH₃)-CH₃, -O-CH₃, -O-CH₂-CH₃, and -CN. Up to two or three heteroatoms may be consecutive, such as, for example, -CH₂-NH-OCH₃ and -CH₂-O-Si(CH₃)₃. A heteroalkyl moiety may include one heteroatom (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include two optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include three optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include four optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include five

optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include up to 8 optionally different heteroatoms (e.g., O, N, S, Si, or P).

[0289] Similarly, the term “heteroalkylene,” by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from heteroalkyl, as exemplified, but not limited by, $-\text{CH}_2-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}_2-$ and $-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}_2-\text{NH}-\text{CH}_2-$. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (e.g., alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula $-\text{C}(\text{O})_2\text{R}'$ represents both $-\text{C}(\text{O})_2\text{R}'$ and $-\text{R}'\text{C}(\text{O})_2-$. As described above, heteroalkyl groups, as used herein, include those groups that are attached to the remainder of the molecule through a heteroatom, such as $-\text{C}(\text{O})\text{R}'$, $-\text{C}(\text{O})\text{NR}'$, $-\text{NR}'\text{R}''$, $-\text{OR}'$, $-\text{SR}'$, and/or $-\text{SO}_2\text{R}'$. Where “heteroalkyl” is recited, followed by recitations of specific heteroalkyl groups, such as $-\text{NR}'\text{R}''$ or the like, it will be understood that the terms heteroalkyl and $-\text{NR}'\text{R}''$ are not redundant or mutually exclusive. Rather, the specific heteroalkyl groups are recited to add clarity. Thus, the term “heteroalkyl” should not be interpreted herein as excluding specific heteroalkyl groups, such as $-\text{NR}'\text{R}''$ or the like.

[0290] The terms “cycloalkyl” and “heterocycloalkyl,” by themselves or in combination with other terms, mean, unless otherwise stated, cyclic versions of “alkyl” and “heteroalkyl,” respectively. Cycloalkyl and heterocycloalkyl are not aromatic. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like. A “cycloalkylene” and a “heterocycloalkylene,” alone or as part of another substituent, means a divalent radical derived from a cycloalkyl and heterocycloalkyl, respectively. “Cycloalkyl” is also meant to refer to bicyclic and polycyclic hydrocarbon rings such as, for example, bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane, etc.

[0291] The terms “halo” or “halogen,” by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as “haloalkyl” are meant to include monohaloalkyl and polyhaloalkyl. For example, the term “halo($\text{C}_1\text{-C}_4$)alkyl” includes, but is not limited to, fluoromethyl, difluoromethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

[0292] The term “acyl” means, unless otherwise stated, -C(O)R where R is a substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

[0293] The term “aryl” means, unless otherwise stated, a polyunsaturated, aromatic, hydrocarbon substituent, which can be a single ring or multiple rings (preferably from 1 to 3 rings) that are fused together (i.e., a fused ring aryl) or linked covalently. A fused ring aryl refers to multiple rings fused together wherein at least one of the fused rings is an aryl ring. The term “heteroaryl” refers to aryl groups (or rings) that contain at least one heteroatom such as N, O, or S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. Thus, the term “heteroaryl” includes fused ring heteroaryl groups (i.e., multiple rings fused together wherein at least one of the fused rings is a heteroaromatic ring). A 5,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 5 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. Likewise, a 6,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. And a 6,5-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 5 members, and wherein at least one ring is a heteroaryl ring. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, naphthyl, pyrrolyl, pyrazolyl, pyridazinyl, triazinyl, pyrimidinyl, imidazolyl, pyrazinyl, purinyl, oxazolyl, isoxazolyl, thiazolyl, furyl, thienyl, pyridyl, pyrimidyl, benzothiazolyl, benzoxazolyl benzimidazolyl, benzofuran, isobenzofuranyl, indolyl, isoindolyl, benzothiophenyl, isoquinolyl, quinoxaliny, quinolyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxaliny, 5-quinoxaliny, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below. An “arylene” and a “heteroarylene,” alone or as part of another substituent, mean a divalent radical derived from an aryl and heteroaryl, respectively. A heteroaryl group substituent may be -O- bonded to a ring heteroatom nitrogen.

[0294] Spirocyclic rings are two or more rings wherein adjacent rings are attached through a single atom. The individual rings within spirocyclic rings may be identical or different.

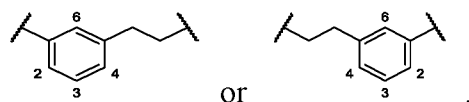
Individual rings in spirocyclic rings may be substituted or unsubstituted and may have different substituents from other individual rings within a set of spirocyclic rings. Possible substituents for individual rings within spirocyclic rings are the possible substituents for the same ring when not part of spirocyclic rings (e.g. substituents for cycloalkyl or heterocycloalkyl rings).

Spirocyclic rings may be substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkyl or substituted or unsubstituted heterocycloalkylene and individual rings within a spirocyclic ring group may be any of the immediately previous list, including having all rings of one type (e.g. all rings being substituted heterocycloalkylene wherein each ring may be the same or different substituted heterocycloalkylene). When referring to a spirocyclic ring system, heterocyclic spirocyclic rings means a spirocyclic rings wherein at least one ring is a heterocyclic ring and wherein each ring may be a different ring. When referring to a spirocyclic ring system, substituted spirocyclic rings means that at least one ring is substituted and each substituent may optionally be different.

[0295] The symbol “” denotes the point of attachment of a chemical moiety to the remainder of a molecule or chemical formula.

[0296] The term “oxo,” as used herein, means an oxygen that is double bonded to a carbon atom.

[0297] The term “alkylarylene” as an arylene moiety covalently bonded to an alkylene moiety (also referred to herein as an alkylene linker). In certain embodiments, the alkylarylene group has the formula:



An alkylarylene moiety may be substituted (e.g. with a substituent group) on the alkylene moiety or the arylene linker (e.g. at carbons 2, 3, 4, or 6) with halogen, oxo, -N₃, -CF₃, -CCl₃, -CBr₃, -Cl₃, -CN, -CHO, -OH, -NH₂, -COOH, -CONH₂, -NO₂, -SH, -SO₂CH₃, -SO₃H, -OSO₃H, -SO₂NH₂, -NHNH₂, -ONH₂, -NHC(O)NHNH₂, substituted or unsubstituted C₁-C₅ alkyl or substituted or unsubstituted 2 to 5 membered heteroalkyl). In certain embodiments, the alkylarylene is unsubstituted.

[0298] Each of the above terms (e.g., “alkyl,” “heteroalkyl,” “cycloalkyl,” “heterocycloalkyl,” “aryl,” and “heteroaryl”) includes both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

[0299] Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to, -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -

SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)₂R', -NR-C(NR'R''R''')=NR''', -NR-C(NR'R'')=NR''', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -NR'NR''R''', -ONR'R'', -NR'C(O)NR''NR''''R''''', -CN, -NO₂, -NR'SO₂R'', -NR'C(O)R'', -NR'C(O)-OR'', -NR'OR'', in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R, R', R'', R''', and R'''' each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl (e.g., aryl substituted with 1-3 halogens), substituted or unsubstituted heteroaryl, substituted or unsubstituted alkyl, alkoxy, or thioalkoxy groups, or arylalkyl groups. When a compound described herein includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''', and R'''' group when more than one of these groups is present. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 4-, 5-, 6-, or 7-membered ring. For example, -NR'R'' includes, but is not limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g., -CF₃ and -CH₂CF₃) and acyl (e.g., -C(O)CH₃, -C(O)CF₃, -C(O)CH₂OCH₃, and the like).

[0300] Similar to the substituents described for the alkyl radical, substituents for the aryl and heteroaryl groups are varied and are selected from, for example: -OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)₂R', -NR-C(NR'R''R''')=NR''', -NR-C(NR'R'')=NR''', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -NR'NR''R''', -ONR'R'', -NR'C(O)NR''NR''''R''''', -CN, -NO₂, -R', -N₃, -CH(Ph)₂, fluoro(C₁-C₄)alkoxy, and fluoro(C₁-C₄)alkyl, -NR'SO₂R'', -NR'C(O)R'', -NR'C(O)-OR'', -NR'OR'', in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R'', R''', and R'''' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. When a compound described herein includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''', and R'''' groups when more than one of these groups is present.

[0301] Substituents for rings (e.g. cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene) may be depicted as substituents on the ring rather than on a specific atom of a ring (commonly referred to as a floating substituent). In such a case, the substituent may be attached to any of the ring atoms (obeying the rules of chemical valency) and in the case of fused rings or spirocyclic rings, a substituent

depicted as associated with one member of the fused rings or spirocyclic rings (a floating substituent on a single ring), may be a substituent on any of the fused rings or spirocyclic rings (a floating substituent on multiple rings). When a substituent is attached to a ring, but not a specific atom (a floating substituent), and a subscript for the substituent is an integer greater than one, the multiple substituents may be on the same atom, same ring, different atoms, different fused rings, different spirocyclic rings, and each substituent may optionally be different. Where a point of attachment of a ring to the remainder of a molecule is not limited to a single atom (a floating substituent), the attachment point may be any atom of the ring and in the case of a fused ring or spirocyclic ring, any atom of any of the fused rings or spirocyclic rings while obeying the rules of chemical valency. Where a ring, fused rings, or spirocyclic rings contain one or more ring heteroatoms and the ring, fused rings, or spirocyclic rings are shown with one more floating substituents (including, but not limited to, points of attachment to the remainder of the molecule), the floating substituents may be bonded to the heteroatoms. Where the ring heteroatoms are shown bound to one or more hydrogens (e.g. a ring nitrogen with two bonds to ring atoms and a third bond to a hydrogen) in the structure or formula with the floating substituent, when the heteroatom is bonded to the floating substituent, the substituent will be understood to replace the hydrogen, while obeying the rules of chemical valency.

[0302] Two or more substituents may optionally be joined to form aryl, heteroaryl, cycloalkyl, or heterocycloalkyl groups. Such so-called ring-forming substituents are typically, though not necessarily, found attached to a cyclic base structure. In one embodiment, the ring-forming substituents are attached to adjacent members of the base structure. For example, two ring-forming substituents attached to adjacent members of a cyclic base structure create a fused ring structure. In another embodiment, the ring-forming substituents are attached to a single member of the base structure. For example, two ring-forming substituents attached to a single member of a cyclic base structure create a spirocyclic structure. In yet another embodiment, the ring-forming substituents are attached to non-adjacent members of the base structure.

[0303] Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally form a ring of the formula $-T-C(O)-(CRR')_q-U-$, wherein T and U are independently $-NR-$, $-O-$, $-CRR'-$, or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-A-(CH_2)_r-B-$, wherein A and B are independently $-CRR'-$, $-O-$, $-NR-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2NR'-$, or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-(CRR')_s-X'-(C''R''R''')_d-$, where s and d are independently integers

of from 0 to 3, and X' is -O-, -NR'-, -S-, -S(O)-, -S(O)₂-, or -S(O)₂NR'-. The substituents R, R', R'', and R''' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

[0304] As used herein, the terms “heteroatom” or “ring heteroatom” are meant to include oxygen (O), nitrogen (N), sulfur (S), phosphorus (P), and silicon (Si).

[0305] A “substituent group,” as used herein, means a group selected from the following moieties:

(A) oxo, halogen, -CF₃, -CN, -OH, -NH₂, -COOH, -CONH₂, -NO₂, -SH, -SO₃H, -SO₄H, -SO₂NH₂, -NHNH₂, -ONH₂, -NHC(=O)NHNH₂, -NHC(=O)NH₂, -NHSO₂H, -NHC(=O)H, -NHC(O)-OH, -NHOH, -OCF₃, -OCHF₂, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(B) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from:

(i) oxo, halogen, -CF₃, -CN, -OH, -NH₂, -COOH, -CONH₂, -NO₂, -SH, -SO₃H, -SO₄H, -SO₂NH₂, -NHNH₂, -ONH₂, -NHC(=O)NHNH₂, -NHC(=O)NH₂, -NHSO₂H, -NHC(=O)H, -NHC(O)-OH, -NHOH, -OCF₃, -OCHF₂, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(ii) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from:

(a) oxo, halogen, -CF₃, -CN, -OH, -NH₂, -COOH, -CONH₂, -NO₂, -SH, -SO₃H, -SO₄H, -SO₂NH₂, -NHNH₂, -ONH₂, -NHC(=O)NHNH₂, -NHC(=O)NH₂, -NHSO₂H, -NHC(=O)H, -NHC(O)-OH, -NHOH, -OCF₃, -OCHF₂, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(b) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from: oxo, halogen, -CF₃, -CN, -OH, -NH₂, -COOH, -CONH₂, -NO₂, -SH, -SO₃H, -SO₄H, -SO₂NH₂, -NHNH₂, -ONH₂, -NHC(=O)NHNH₂, -NHC(=O)NH₂, -NHSO₂H, -NHC(=O)H, -NHC(O)-OH, -NHOH, -OCF₃, -OCHF₂, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl.

[0306] A “size-limited substituent” or “ size-limited substituent group,” as used herein, means a group selected from all of the substituents described above for a “substituent group,” wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C₁-C₂₀ alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C₃-C₈ cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C₆-C₁₀ aryl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl.

[0307] A “lower substituent” or “ lower substituent group,” as used herein, means a group selected from all of the substituents described above for a “substituent group,” wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C₁-C₈ alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C₃-C₇ cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C₆-C₁₀ aryl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 9 membered heteroaryl.

[0308] In some certain embodiments, each substituted group described in the compounds herein is substituted with at least one substituent group. More specifically, in some certain embodiments, each substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylenes, and/or substituted heteroarylenes described in the compounds herein are substituted with at least one substituent group. In other certain embodiments, at least one or all of these groups are substituted with at least one size-limited substituent group. In other certain embodiments, at least one or all of these groups are substituted with at least one lower substituent group.

[0309] In other embodiments of the compounds herein, each substituted or unsubstituted alkyl may be a substituted or unsubstituted C₁-C₂₀ alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C₃-C₈ cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C₆-C₁₀ aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl. In some embodiments of the compounds herein, each substituted or unsubstituted

alkylene is a substituted or unsubstituted C₁-C₂₀ alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 20 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C₃-C₈ cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 3 to 8 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C₆-C₁₀ arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 10 membered heteroarylene.

[0310] In some certain embodiments, each substituted or unsubstituted alkyl is a substituted or unsubstituted C₁-C₈ alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C₃-C₇ cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C₆-C₁₀ aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 9 membered heteroaryl. In some certain embodiments, each substituted or unsubstituted alkylene is a substituted or unsubstituted C₁-C₈ alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 8 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C₃-C₇ cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 3 to 7 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C₆-C₁₀ arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 9 membered heteroarylene. In some certain embodiments, the compound is a chemical species set forth in the Examples section, figures, or tables below.

[0311] Certain compounds of the present invention possess asymmetric carbon atoms (optical or chiral centers) or double bonds; the enantiomers, racemates, diastereomers, tautomers, geometric isomers, stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)- or, as (D)- or (L)- for amino acids, and individual isomers are encompassed within the scope of the present invention. The compounds of the present invention do not include those that are known in art to be too unstable to synthesize and/or isolate. The present invention is meant to include compounds in racemic and optically pure forms. Optically active (R)- and (S)-, or (D)- and (L)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers.

[0312] As used herein, the term “isomers” refers to compounds having the same number and kind of atoms, and hence the same molecular weight, but differing in respect to the structural arrangement or configuration of the atoms.

[0313] The term “tautomer,” as used herein, refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one isomeric form to another.

[0314] It will be apparent to one skilled in the art that certain compounds of this invention may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the invention.

[0315] Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the invention.

[0316] Unless otherwise stated, structures depicted herein are also meant to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by ^{13}C - or ^{14}C -enriched carbon are within the scope of this invention.

[0317] The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^3H), iodine-125 (^{125}I), or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are encompassed within the scope of the present invention.

[0318] It should be noted that throughout the application that alternatives are written in Markush groups, for example, each amino acid position that contains more than one possible amino acid. It is specifically contemplated that each member of the Markush group should be considered separately, thereby comprising another embodiment, and the Markush group is not to be read as a single unit.

[0319] “Analog” or “analogue” is used in accordance with its plain ordinary meaning within Chemistry and Biology and refers to a chemical compound that is structurally similar to another compound (i.e., a so-called “reference” compound) but differs in composition, e.g., in the replacement of one atom by an atom of a different element, or in the presence of a particular functional group, or the replacement of one functional group by another functional group, or the absolute stereochemistry of one or more chiral centers of the reference compound. Accordingly,

an analog is a compound that is similar or comparable in function and appearance but not in structure or origin to a reference compound.

[0320] The terms “a” or “an,” as used in herein means one or more. In addition, the phrase “substituted with a[n],” as used herein, means the specified group may be substituted with one or more of any or all of the named substituents. For example, where a group, such as an alkyl or heteroaryl group, is “substituted with an unsubstituted C₁-C₂₀ alkyl, or unsubstituted 2 to 20 membered heteroalkyl,” the group may contain one or more unsubstituted C₁-C₂₀ alkyls, and/or one or more unsubstituted 2 to 20 membered heteroalkyls.

[0321] Moreover, where a moiety is substituted with an R substituent, the group may be referred to as “R-substituted.” Where a moiety is R-substituted, the moiety is substituted with at least one R substituent and each R substituent is optionally different. Where a particular R group is present in the description of a chemical genus (such as Formula (II)), a Roman alphabetic symbol may be used to distinguish each appearance of that particular R group. For example, where multiple R¹³ substituents are present, each R¹³ substituent may be distinguished as R^{13A}, R^{13B}, R^{13C}, R^{13D}, etc., wherein each of R^{13A}, R^{13B}, R^{13C}, R^{13D}, etc. is defined within the scope of the definition of R¹³ and optionally differently.

[0322] Descriptions of compounds of the present invention are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds which are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions, such as aqueous, neutral, and several known physiological conditions. For example, a heterocycloalkyl or heteroaryl is attached to the remainder of the molecule via a ring heteroatom in compliance with principles of chemical bonding known to those skilled in the art thereby avoiding inherently unstable compounds.

[0323] The term “pharmaceutically acceptable salts” is meant to include salts of the active compounds that are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of

pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, oxalic, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (*see*, for example, Berge *et al.*, “Pharmaceutical Salts”, *Journal of Pharmaceutical Science*, **1977**, 66, 1-19). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

[0324] Thus, the compounds of the present invention may exist as salts, such as with pharmaceutically acceptable acids. The present invention includes such salts. Non-limiting examples of such salts include hydrochlorides, hydrobromides, phosphates, sulfates, methanesulfonates, nitrates, maleates, acetates, citrates, fumarates, propionates, tartrates (e.g., (+)-tartrates, (-)-tartrates, or mixtures thereof including racemic mixtures), succinates, benzoates, and salts with amino acids such as glutamic acid, and quaternary ammonium salts (e.g. methyl iodide, ethyl iodide, and the like). These salts may be prepared by methods known to those skilled in the art.

[0325] The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound may differ from the various salt forms in certain physical properties, such as solubility in polar solvents. In certain embodiments, compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts. The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in a conventional manner. The parent form of the compounds differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but, unless specifically indicated, the salts disclosed herein are equivalent to the parent form of the compound for the purposes of the present invention.

[0326] In addition to salt forms, the present invention provides compounds, which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Prodrugs of the compounds described herein may be converted *in vivo* after administration. Additionally, prodrugs can be converted to the compounds of the present

invention by chemical or biochemical methods in an *ex vivo* environment, such as, for example, when contacted with a suitable enzyme or chemical reagent.

[0327] Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

[0328] “Contacting” is used in accordance with its plain ordinary meaning and refers to the process of allowing at least two distinct species (e.g. chemical compounds including biomolecules or cells) to become sufficiently proximal to react, interact or physically touch. It should be appreciated; however, the resulting reaction product can be produced directly from a reaction between the added reagents or from an intermediate from one or more of the added reagents that can be produced in the reaction mixture.

[0329] The term “contacting” may include allowing two species to react, interact, or physically touch, wherein the two species may be a compound as described herein and a protein or enzyme. In some embodiments contacting includes allowing a compound described herein to interact with a protein or enzyme that is involved in a signaling pathway.

[0330] As defined herein, the term “activation”, “activate”, “activating” and the like in reference to a protein refers to conversion of a protein into a biologically active derivative from an initial inactive or deactivated state. The terms reference activation, or activating, sensitizing, or up-regulating signal transduction or enzymatic activity or the amount of a protein decreased in a disease.

[0331] The terms “agonist,” “activator,” “upregulator,” etc. refer to a substance capable of detectably increasing the expression or activity of a given gene or protein. The agonist can increase expression or activity 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a control in the absence of the agonist. In certain instances, expression or activity is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or higher than the expression or activity in the absence of the agonist. In certain embodiments, an agonist is a molecule that interacts with a target to cause or promote an increase in the activation of the target. In certain embodiments, activators are molecules that increase, activate, facilitate, enhance activation, sensitize, or up-regulate, e.g., a gene, protein, ligand, receptor, or cell.

[0332] As defined herein, the term “inhibition,” “inhibit,” “inhibiting,” and the like, in reference to a protein-inhibitor interaction means negatively affecting (e.g. decreasing) the activity or function of the protein relative to the activity or function of the protein in the absence

of the inhibitor. In embodiments inhibition means negatively affecting (e.g. decreasing) the concentration or levels of the protein relative to the concentration or level of the protein in the absence of the inhibitor. In embodiments inhibition refers to reduction of a disease or symptoms of disease. In certain embodiments, inhibition refers to a reduction in the activity of a particular protein target. Thus, inhibition includes, at least in part, partially or totally blocking stimulation, decreasing, preventing, or delaying activation, or inactivating, desensitizing, or down-regulating signal transduction or enzymatic activity or the amount of a protein. In certain embodiments, inhibition refers to a reduction of activity of a target protein resulting from a direct interaction (e.g. an inhibitor binds to the target protein). In certain embodiments, inhibition refers to a reduction of activity of a target protein from an indirect interaction (e.g. an inhibitor binds to a protein that activates the target protein, thereby preventing target protein activation).

[0333] The terms “inhibitor,” “repressor” or “antagonist” or “downregulator” interchangeably refer to a substance capable of detectably decreasing the expression or activity of a given gene or protein. The antagonist can decrease expression or activity 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a control in the absence of the antagonist. In certain instances, expression or activity is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or lower than the expression or activity in the absence of the antagonist. An antagonist prevents, reduces, inhibits, or neutralizes the activity of an agonist, and an antagonist can also prevent, inhibit, or reduce constitutive activity of a target, e.g., a target receptor, even where there is no identified agonist. In certain embodiments, inhibitors are molecules that decrease, block, prevent, delay activation, inactivate, desensitize, or down-regulate, e.g., a gene, protein, ligand, receptor, or cell. An inhibitor may also be defined as a molecule that reduces, blocks, or inactivates a constitutive activity. An “antagonist” is a molecule that opposes the action(s) of an agonist.

[0334] The terms “disease” or “condition” refer to a state of being or health status of a patient or subject capable of being treated with the compounds or methods provided herein. The disease may be a cancer.

[0335] The terms “treating” or “treatment” refer to any indicia of success in the therapy or amelioration of an injury, disease, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the patient; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a patient’s physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, neuropsychiatric exams, and/or a psychiatric evaluation. The term “treating” and conjugations thereof, may include

prevention of an injury, pathology, condition, or disease. In certain embodiments, treating is preventing. In certain embodiments, treating does not include preventing.

[0336] “Treating” or “treatment” as used herein (and as well-understood in the art) also broadly includes any approach for obtaining beneficial or desired results in a subject’s condition, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of the extent of a disease, stabilizing (*i.e.*, not worsening) the state of disease, prevention of a disease’s transmission or spread, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission, whether partial or total and whether detectable or undetectable. In other words, “treatment” as used herein includes any cure, amelioration, or prevention of a disease. Treatment may prevent the disease from occurring; inhibit the disease’s spread; relieve the disease’s symptoms, fully or partially remove the disease’s underlying cause, shorten a disease’s duration, or do a combination of these things.

[0337] “Treating” and “treatment” as used herein include prophylactic treatment. Treatment methods include administering to a subject a therapeutically effective amount of a compound described herein. The administering step may consist of a single administration or may include a series of administrations. The length of the treatment period depends on a variety of factors, such as the severity of the condition, the age of the patient, the concentration of the compound, the activity of the compositions used in the treatment, or a combination thereof. It will also be appreciated that the effective dosage of an agent used for the treatment or prophylaxis may increase or decrease over the course of a particular treatment or prophylaxis regime. Changes in dosage may result and become apparent by standard diagnostic assays known in the art. In some instances, chronic administration may be required. For example, the compositions are administered to the subject in an amount and for a duration sufficient to treat the patient.

[0338] The term “prevent” refers to a decrease in the occurrence of disease symptoms in a patient. As indicated above, the prevention may be complete (no detectable symptoms) or partial, such that fewer symptoms are observed than would likely occur absent treatment. In certain embodiments, prevent refers to slowing the progression of the disease, disorder or condition or inhibiting progression thereof to a harmful or otherwise undesired state.

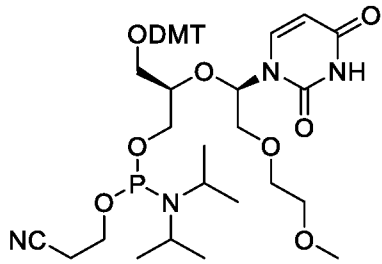
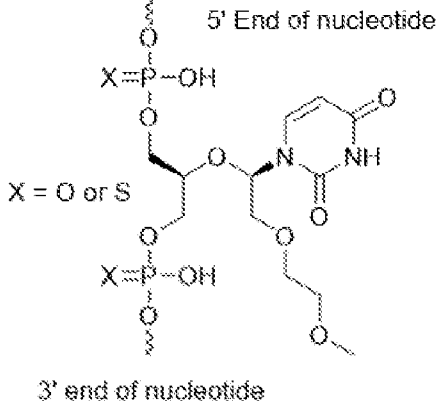
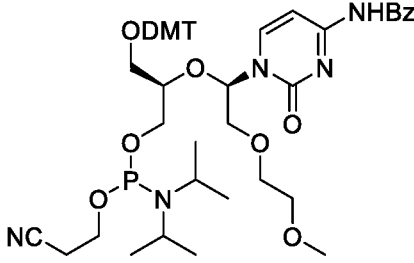
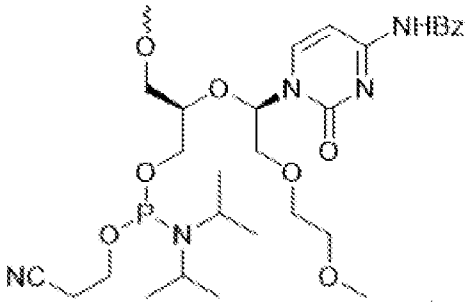
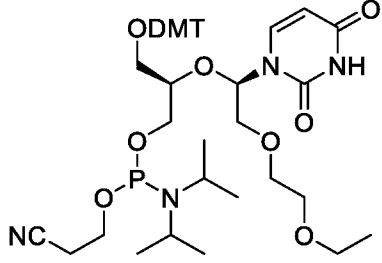
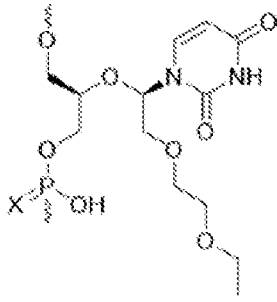
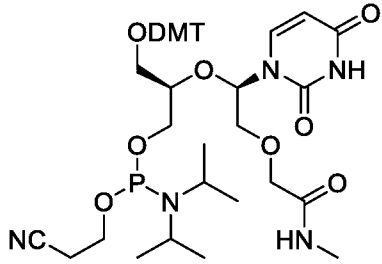
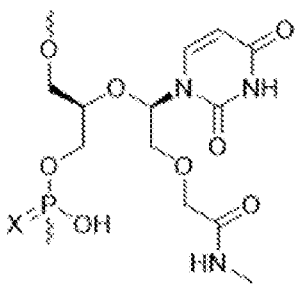
EXAMPLES

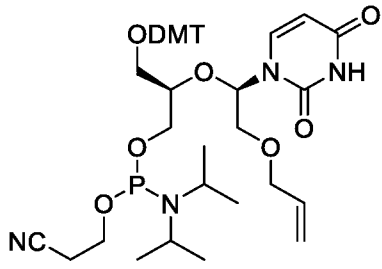
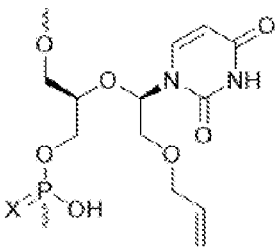
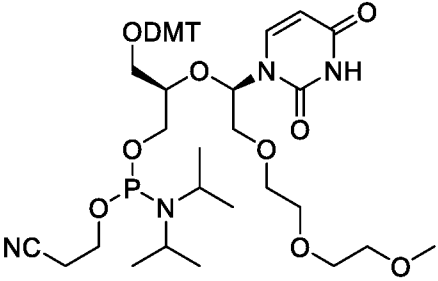
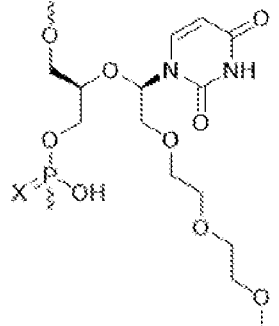
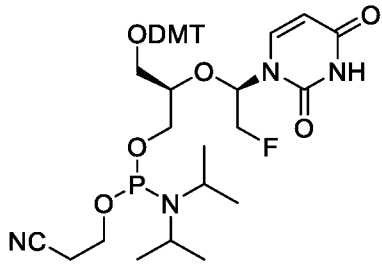
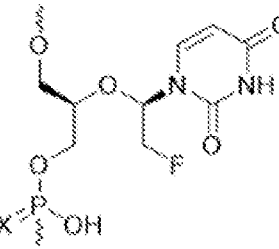
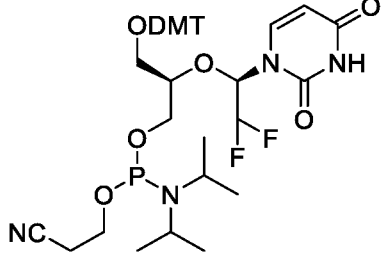
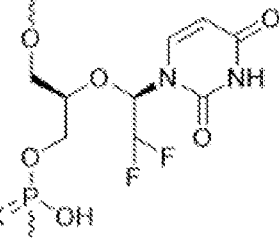
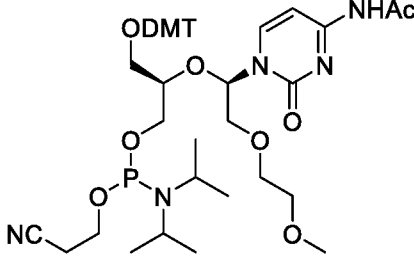
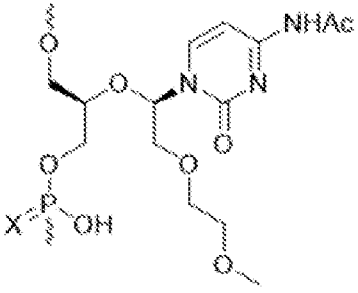
[0339] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

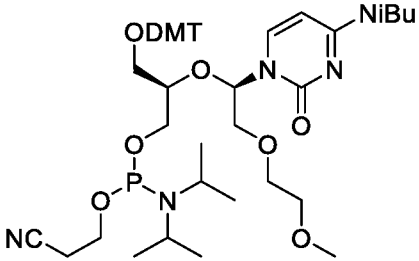
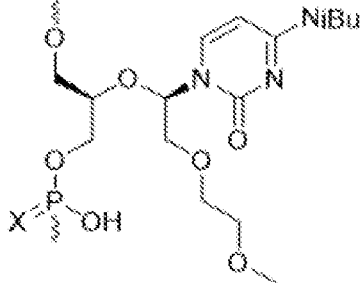
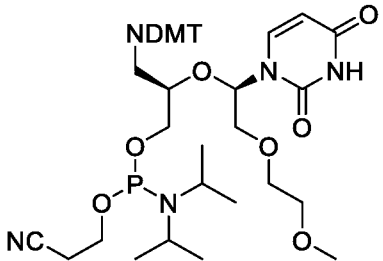
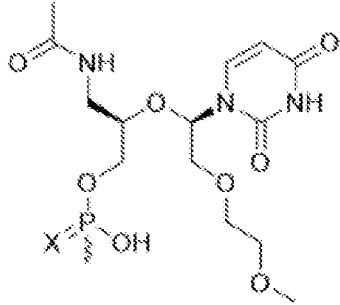
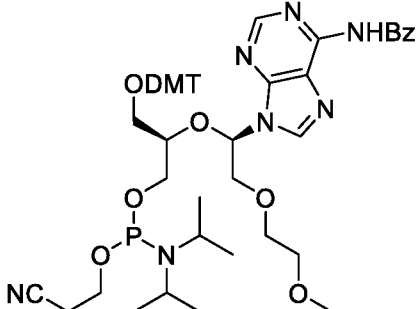
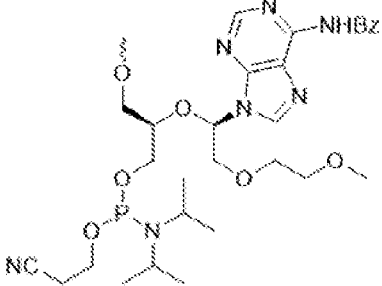
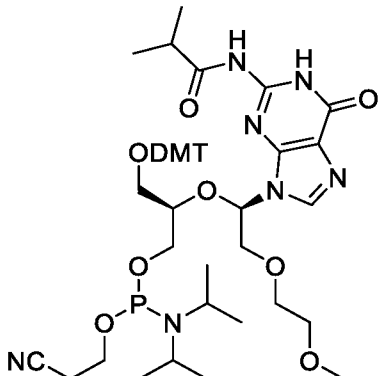
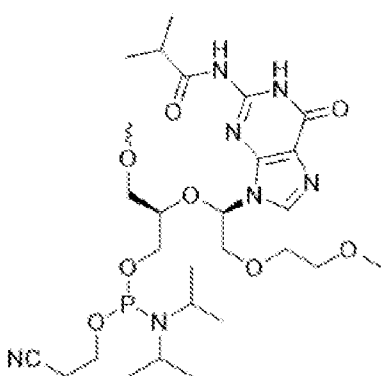
Chemical Synthesis Examples

[0340] The compounds in Tables 1-3 are prepared in the following examples.

TABLE 1

Compound/ Eg No	UNA Structure	UNA Structure as a part of oligonucleotide
1		 5' End of nucleotide X = O or S 3' end of nucleotide
2		
3		
4		

Compound/ Eg No	UNA Structure	UNA Structure as a part of oligonucleotide
5		
6		
7		
8		
9		

Compound/ Eg No	UNA Structure	UNA Structure as a part of oligonucleotide
10		
11		
12		
13		

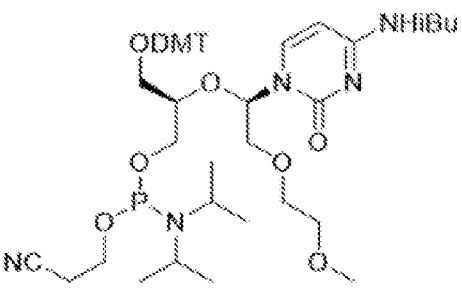
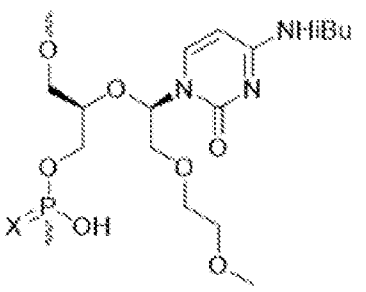
Compound/ Eg No	UNA Structure	UNA Structure as a part of oligonucleotide
14		

TABLE 2

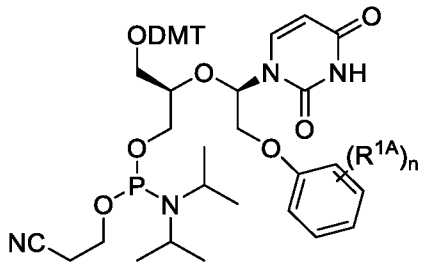
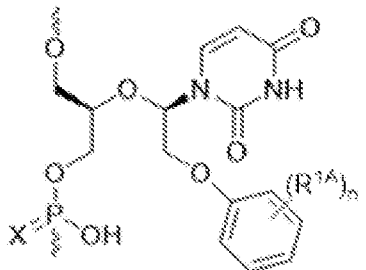
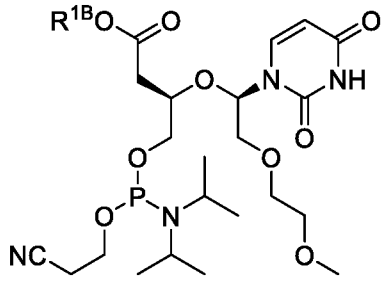
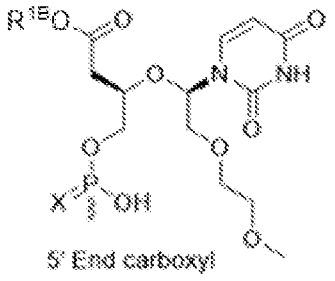
Compound/ Eg No	Structure	UNA Structure as a part of oligonucleotide
15		

TABLE 3

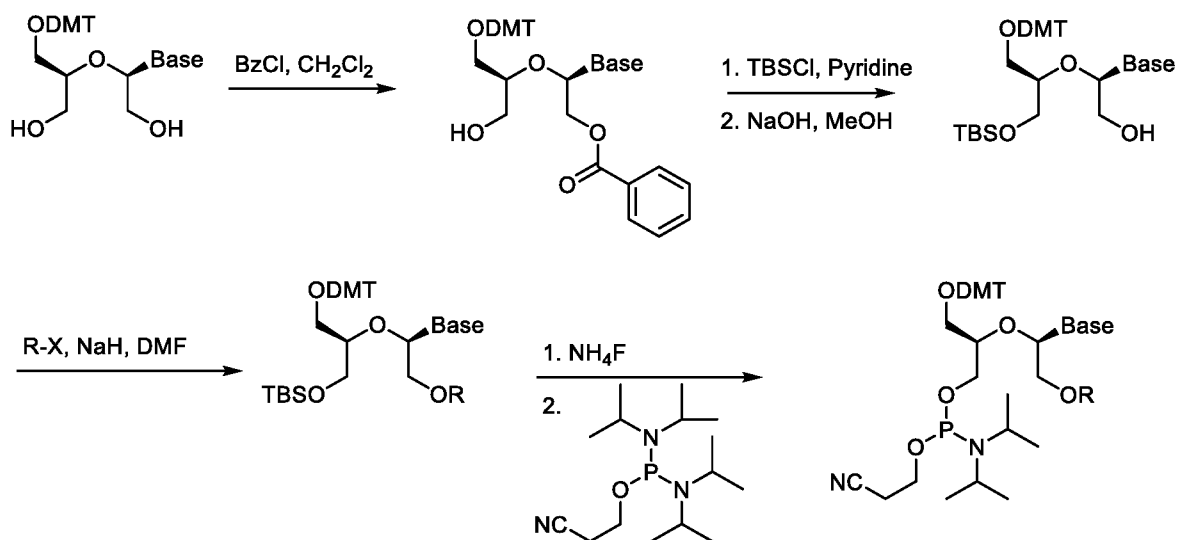
Compound/ Eg No	Structure	UNA Structure as a part of oligonucleotide
16		

Example 1. General synthetic scheme for the preparation of Compounds 1-13, UNA 2'-derivatives:

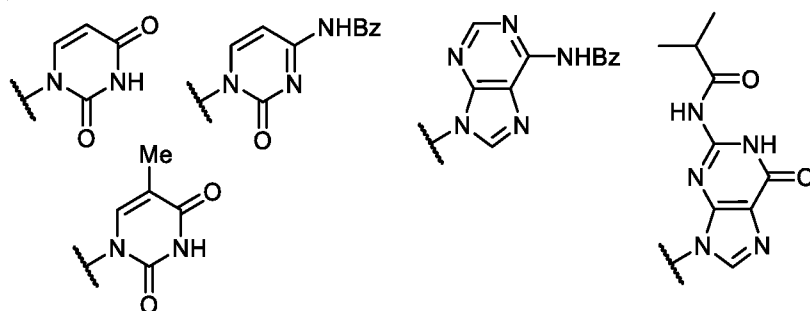
[0341] Compounds 1-15 are synthesized according to the following schemes.

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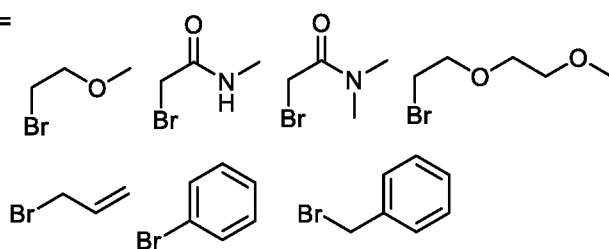
PCT/US2020/036420



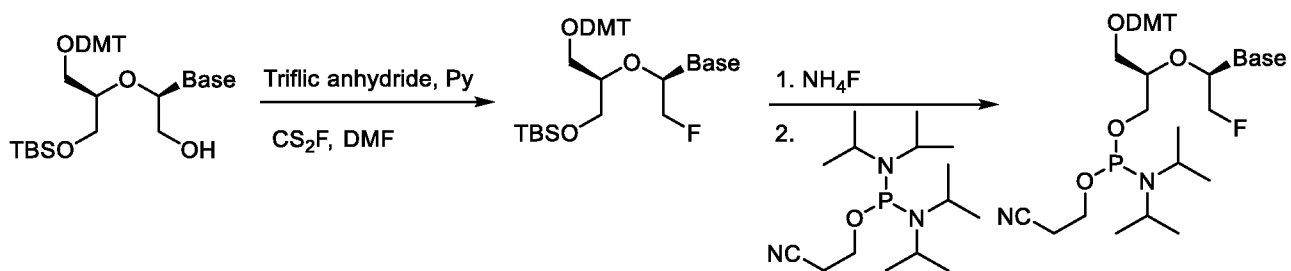
Base =



R-X =

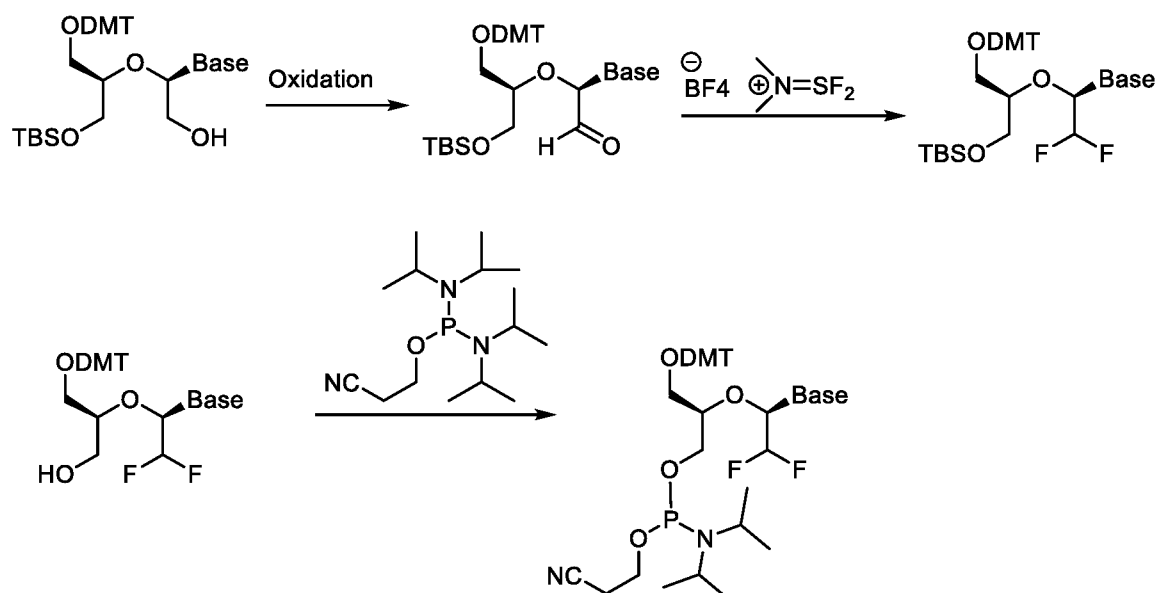
**Example 2. General Synthetic Scheme for UNA 2' Fluoro Compounds:**

[0342] Compounds 2 and 8 are synthesized according to the following schemes.

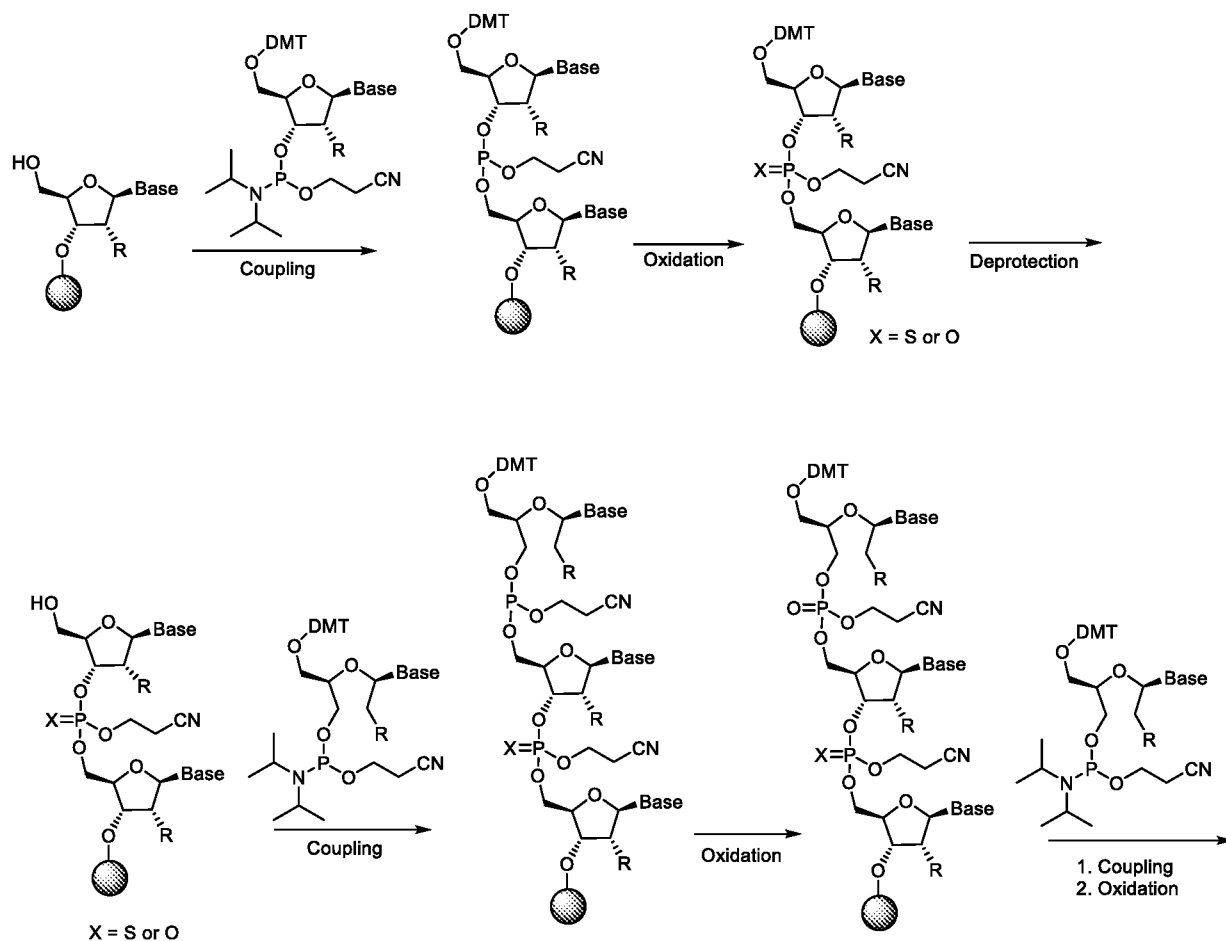


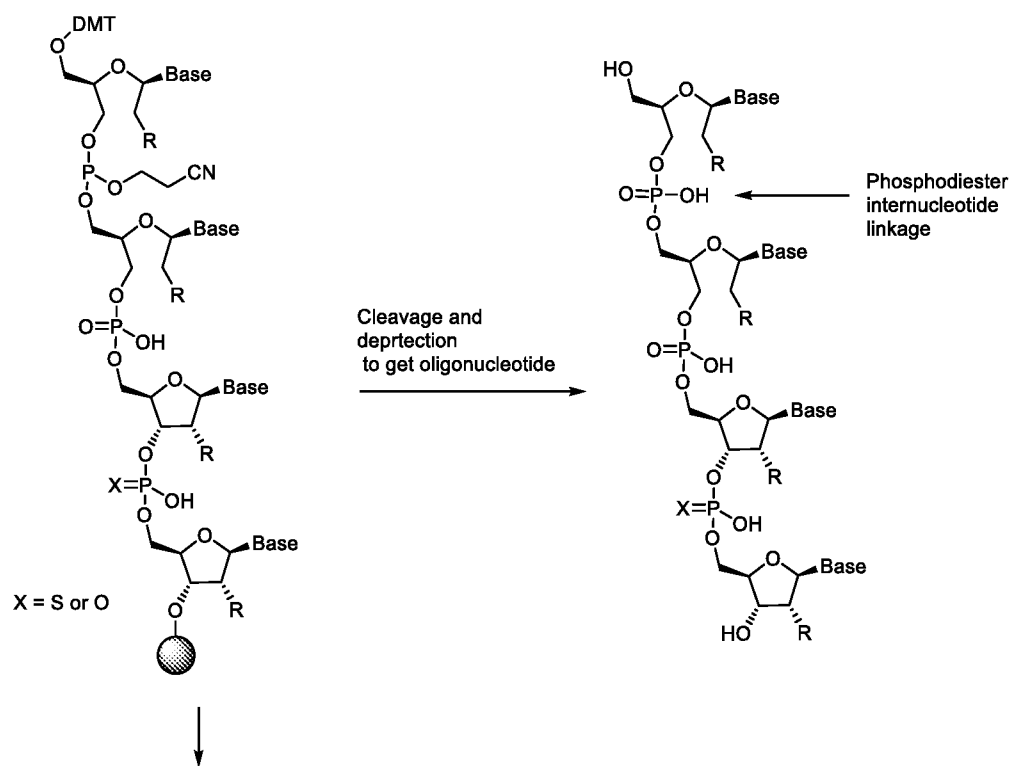
WO 2020/247818

PCT/US2020/036420



Example 3. General Synthetic Scheme for the Solid Phase Synthesis of Oligonucleotides:



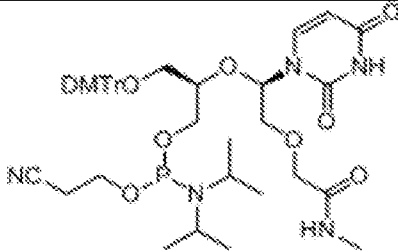
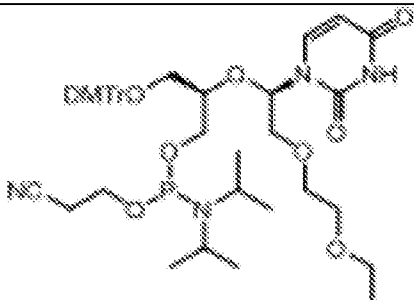


Oligonucleotide could be further lengthened by repeating additional cycles of coupling and oxidation

Example 4. UNA key

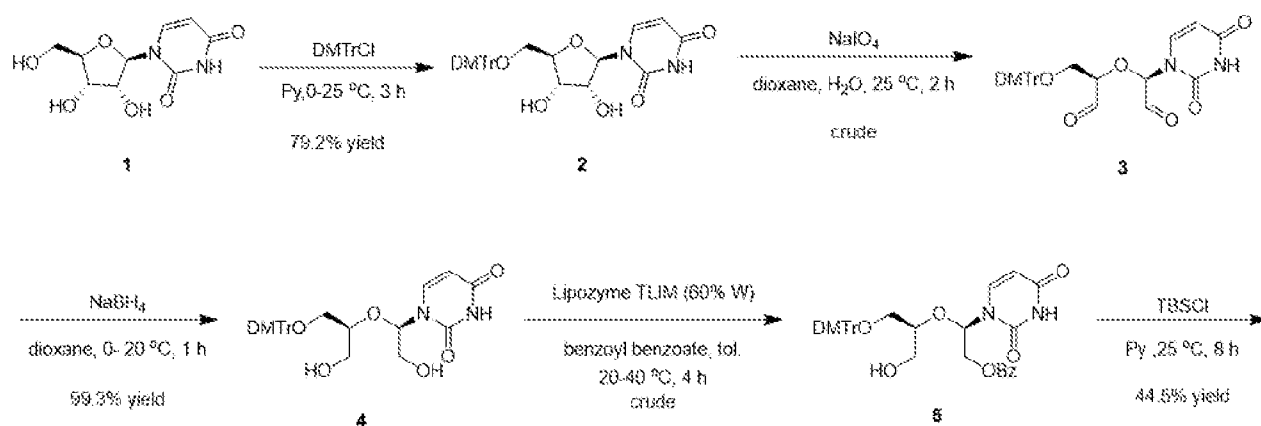
TABLE 4

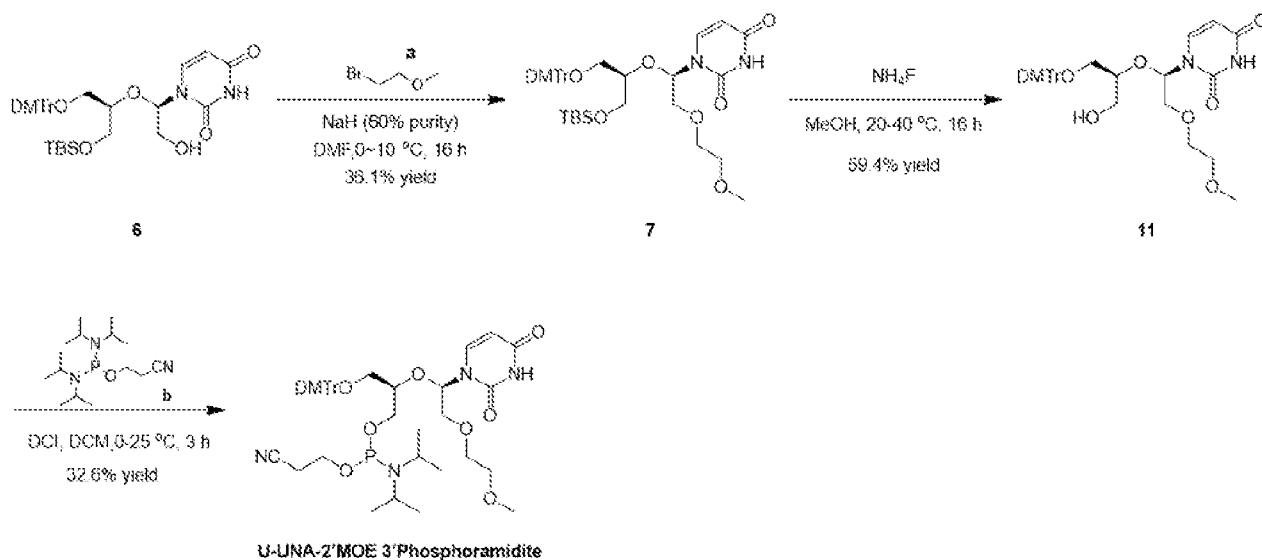
Structure	Modification Name	Code
The structure shows a nucleoside with a 2'-MOE (2-methoxyethyl) modification. The 5' position is protected with a DMT group. The 3' position is linked to a phosphoramidite group via a phosphite triester linkage. The phosphoramidite group consists of a phosphorus atom double-bonded to an oxygen atom and single-bonded to a hydroxyl group and an amino group (X = S or O). The amino group is further substituted with a hydroxymethyl group. The phosphite triester linkage is shown as a phosphorus atom double-bonded to an oxygen atom and single-bonded to two other oxygen atoms, one of which is part of the nucleoside sugar ring. The other oxygen atom is part of a hydroxymethyl group. The phosphite triester linkage is labeled as a "Phosphodiester internucleotide linkage".	UNA with 2'-MOE	Uq
The structure shows a nucleoside with a 2'-EOE (2-ethoxyethyl) modification. The 5' position is protected with a DMT group. The 3' position is linked to a phosphoramidite group via a phosphite triester linkage. The phosphoramidite group consists of a phosphorus atom double-bonded to an oxygen atom and single-bonded to a hydroxyl group and an amino group (X = S or O). The amino group is further substituted with a hydroxymethyl group. The phosphite triester linkage is shown as a phosphorus atom double-bonded to an oxygen atom and single-bonded to two other oxygen atoms, one of which is part of the nucleoside sugar ring. The other oxygen atom is part of a hydroxymethyl group. The phosphite triester linkage is labeled as a "Phosphodiester internucleotide linkage".	UNA with 2'-EOE	Uw

	UNA with 2'-methyl acetamide	Ux
	UNA with 2'-mPEG2	Uy

Example 5. Synthesis of Uridine-UNA-2'MOE, 3'Phosphoramidite

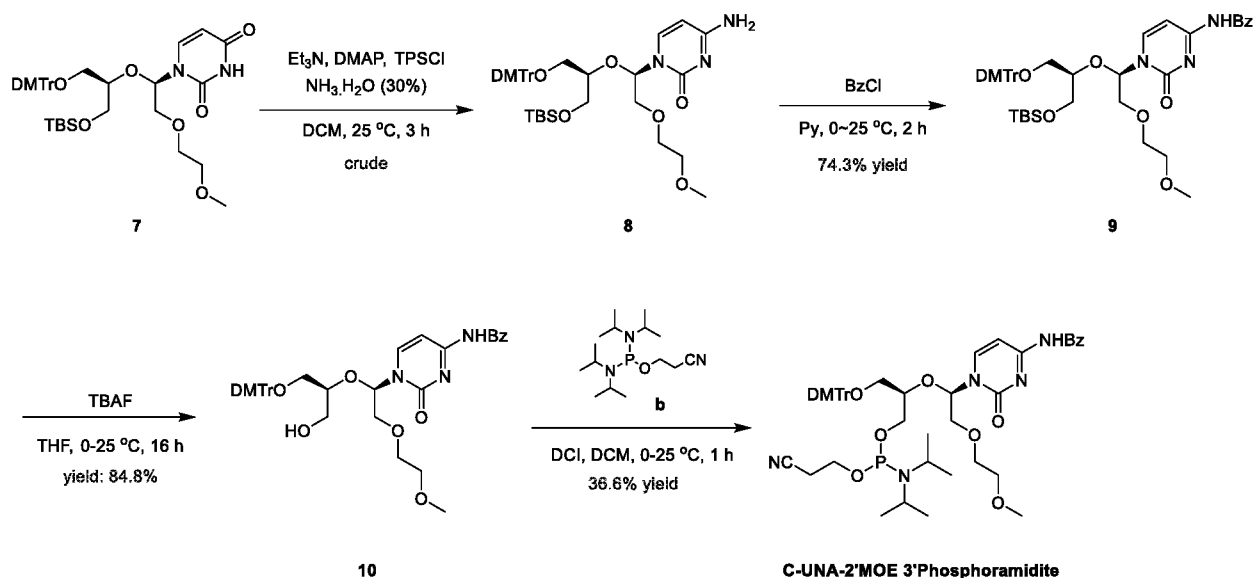
[0343] Uridine-UNA-2'MOE, 3'Phosphoramidite is synthesized according to the following schemes.





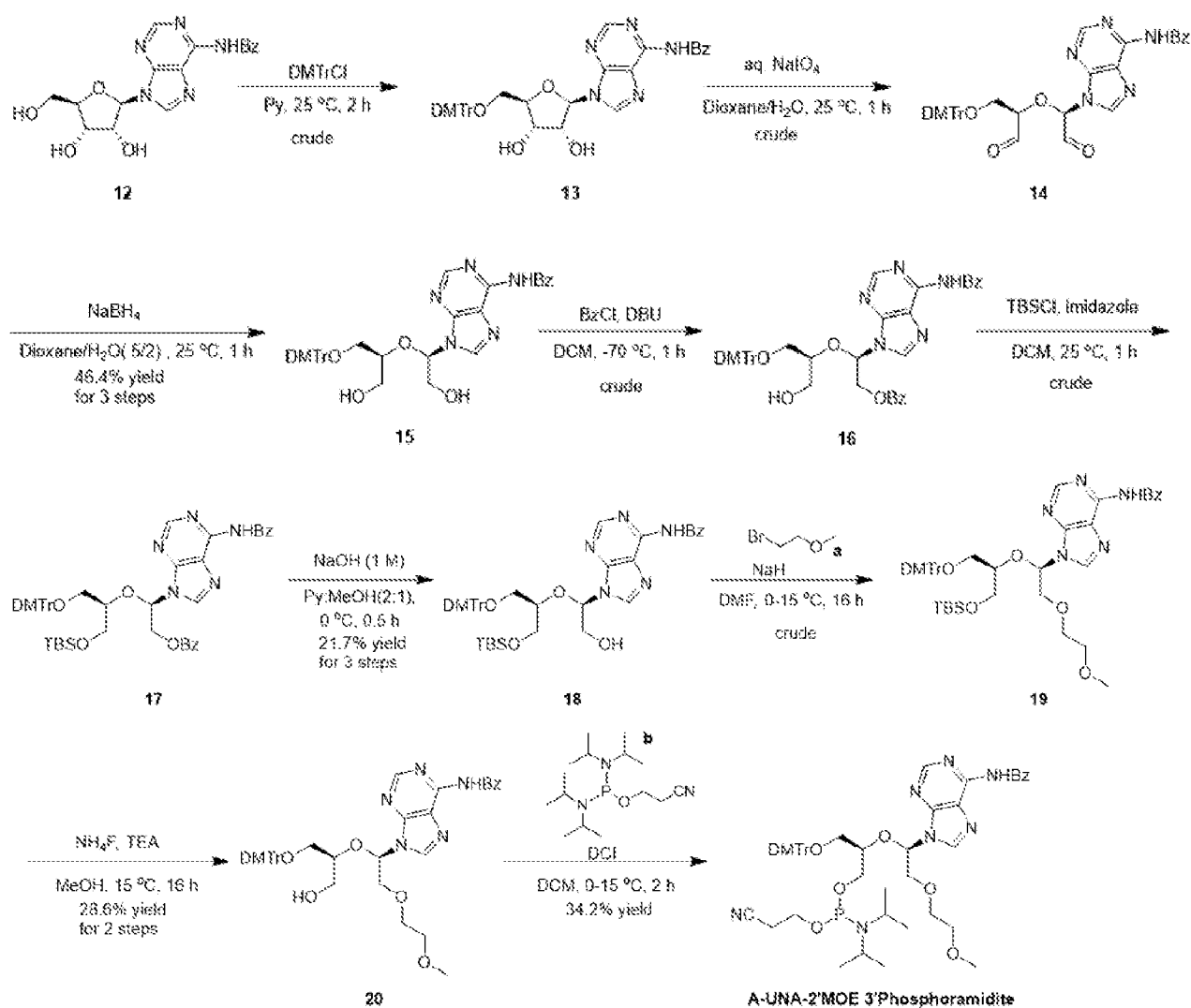
Example 6. Synthesis of Cytosine-UNA-2'MOE, 3'Phosphoramidite

[0344] Cytosine-UNA-2'MOE, 3'Phosphoramidite is synthesized according to the following schemes.



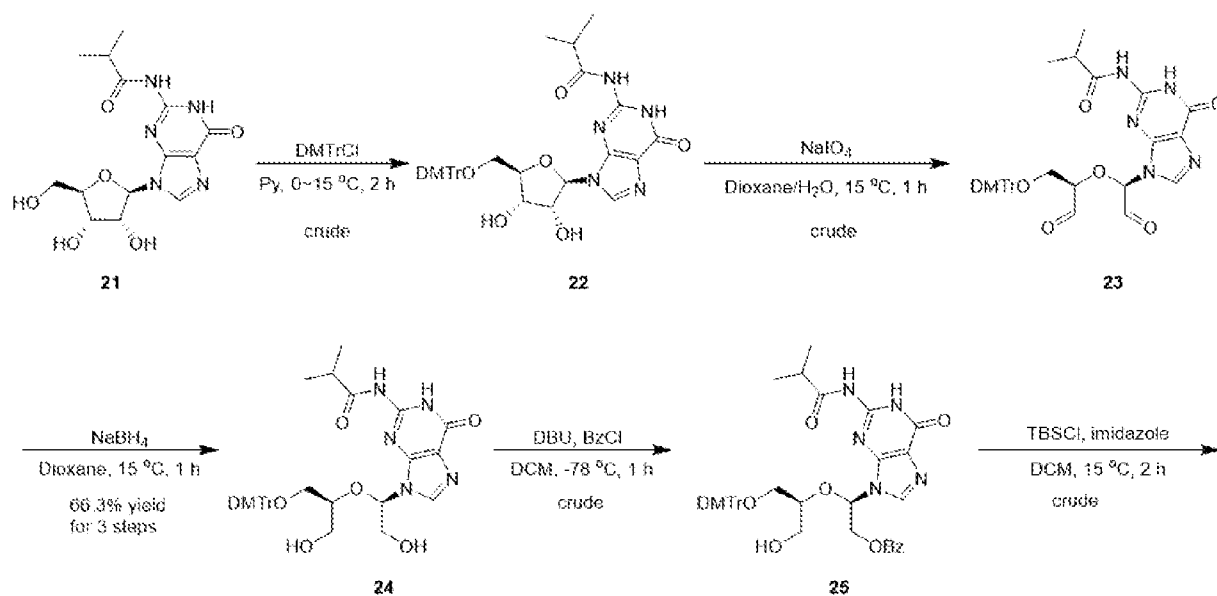
Example 7. Synthesis of Adenine-UNA-2'MOE, 3'Phosphoramidite

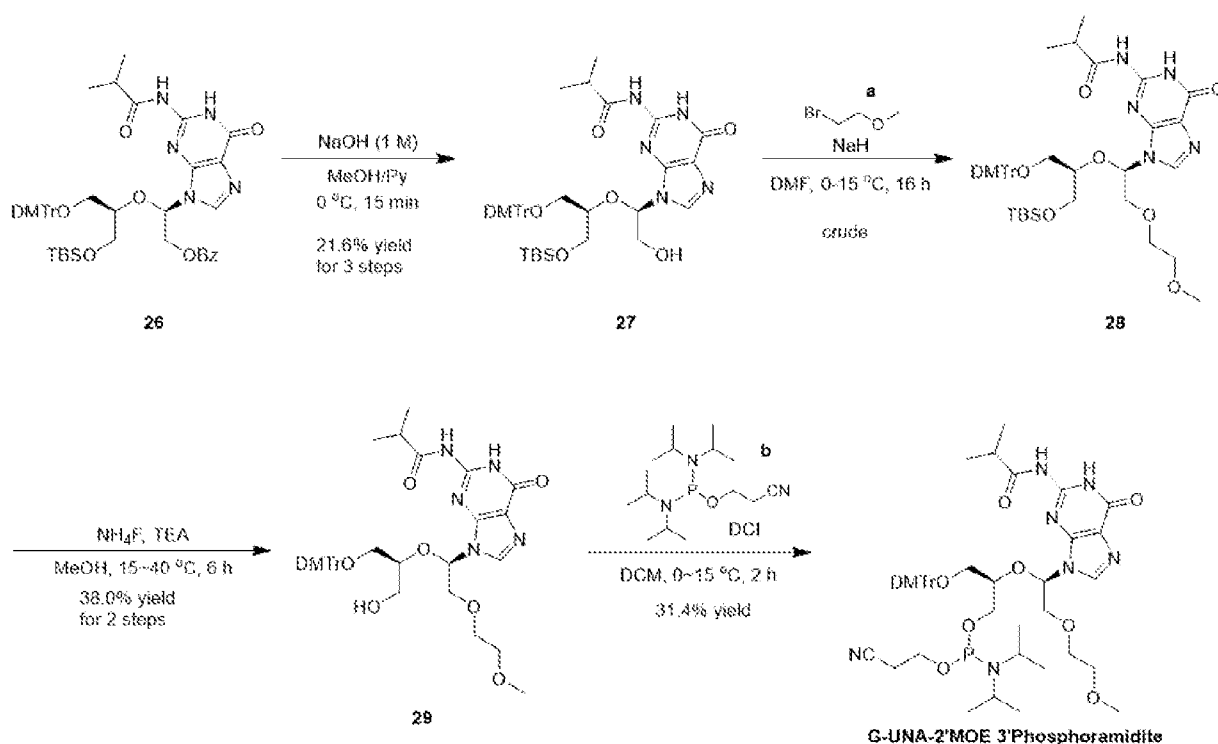
[0345] Adenine -UNA-2'MOE, 3'Phosphoramidite is synthesized according to the following schemes.



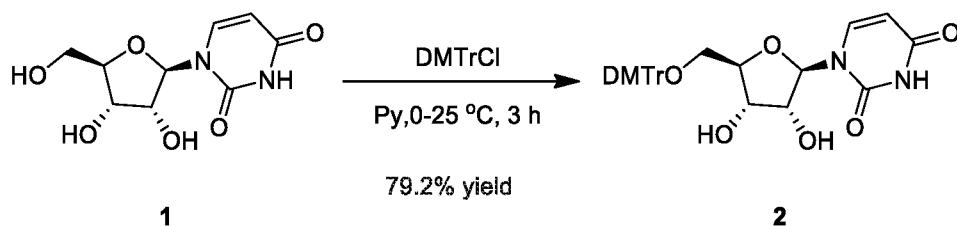
Example 8. Synthesis of Guanosine-UNA-2'MOE, 3'Phosphoramidite

[0346] Guanosine -UNA-2'MOE, 3'Phosphoramidite is synthesized according to the following schemes.



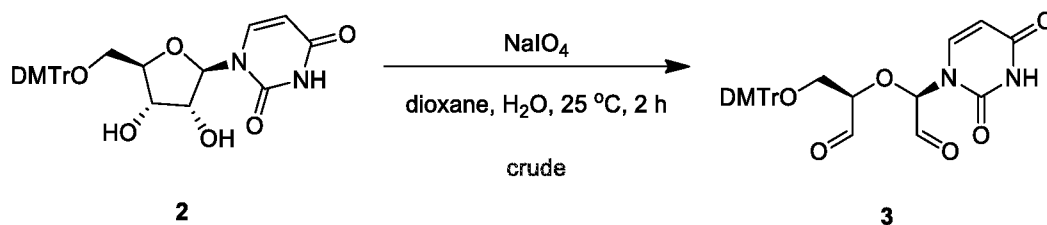


Example 9. General procedure for preparation of compound 2



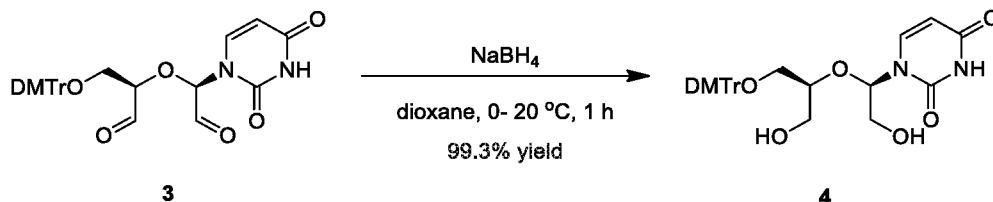
[0347] To a solution of compound 1 (350 g, 1.43 mol, 1.00 eq) in Py (2.1 L), was added DMTCl (500.2 g, 1.48 mol, 1.03 eq) at 0 °C. The reaction mixture was stirred at 25 °C for 3 h. TLC (Dichloromethane: Methanol = 10: 1, R_f = 0.51) showed no starting material. The mixture was diluted with aq. NaHCO₃ (2.0 L) and extracted with DCM (2.0 L x 2), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. Concentrate the combined organic layer to give the crude product. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate = 10/1 to 0/1 and 0.5% TEA). Afforded compound 2 (620 g, 1.13 mol, 79.2 yield) as yellow solid.

Example 10. General procedure for preparation of compound 3



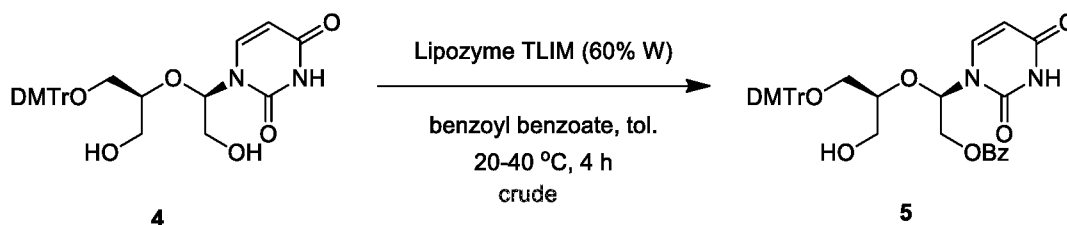
[0348] To a solution of compound **2** (310 g, 567.2 mmol, 1.00 *eq*) in dioxane (1.5 L), was added NaIO₄ (121.3 g, 567.2 mmol, 31.4 mL, 1.00 *eq*) and H₂O (600 mL) at 25 °C, the reaction mixture was stirred at 25 °C for 2 h. TLC (Dichloromethane: Methanol = 10: 1, R_f = 0.64) showed starting material was consumed completely. The two reactions were combined for workup. The reaction mixture was filtered. The crude product was used into the next step without further purification. Afforded compound **3** (600 g, crude) as a colorless oil.

Example 11. General procedure for preparation of compound **4**



[0349] To a solution of compound **3** (300 g, 550.9 mmol, 1 *eq*) in dioxane (1.8 L), was added NaBH₄ (20.8 g, 550.9 mmol, 1.00 *eq*) at 0 °C, the mixture was stirred at 20 °C for 1 h. TLC (Dichloromethane: Methanol = 10: 1, R_f = 0.43) showed no starting material. The two reactions were combined for workup; the reaction mixture was quenched by acetone, neutralized with 20% acetic acid aq., and concentrated to give a residue. The residue was diluted with DCM (1.8 L), and washed with H₂O (1.8 L), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Afforded compound **4** (600 g, 1.09 mol, 99.3% yield) as white solid.

Example 12. General procedure for preparation of compound **5**



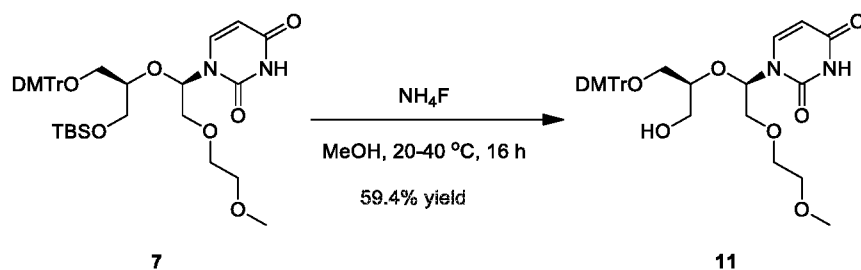
[0350] To a solution of compound **4** (300 g, 546.9 mmol, 1.00 *eq*) in Tol. (600 mL) was added lipzyme TL IM (60 g, 0.60 *eq*) and Benzoyl benzoate (124.9 g, 552.3 mmol, 104.1 mL, 1.01 *eq*) at 20 °C. The reaction mixture was stirred at 40 °C for 4 h. TLC (Dichloromethane: Methanol = 10: 1, R_f = 0.53) showed no starting material. Combined the two reactions for workup, the reaction mixture was filtered, the filter liquor was partitioned between aqueous NaHCO₃ (600 mL) and EtOAc (600 mL). The organic phase was separated, washed with brine (600 mL x 3), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Afforded compound **5** (620 g, crude) as white solid.

were purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate = 10/1 to 0:1 and 0.5% TEA). Afforded compound **7** (110 g, 152.6 mmol, 36.1% yield) as yellow oil.

[0353] ¹H NMR: ET32436-23-P1A (400 MHz, DMSO-*d*₆)

[0354] δ 11.37 (s, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.33-7.30 (m, 4H), 7.19-7.16 (m, 5H), 6.86-6.84 (m, 4H), 6.00 (t, *J* = 4.8 Hz, 1H), 5.55 (d, *J* = 8.0 Hz, 1H), 3.73 (s, 6H), 3.71-3.69 (m, 2H), 3.65-3.63 (m, 2H), 3.56-3.55 (m, 3H), 3.41-3.39 (m, 2H), 3.21 (s, 3H), 2.97-2.95 (m, 2H), -0.76 (s, 9H), -0.04 (d, *J* = 7.6 Hz, 6.0H)

Example 15. General procedure for preparation of compound **11**

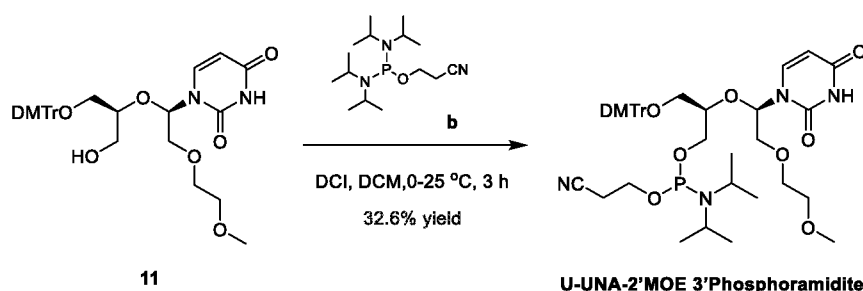


[0355] To a solution of compound **7** (15 g, 20.8 mmol, 1.00 *eq*) in MeOH (120 mL) was added NH₄F (6.17 g, 166.5 mmol, 8.00 *eq*) at 20 °C, the reaction mixture was stirred at 40 °C for 16 h. TLC (Petroleum ether: Ethyl acetate = 0:1, R_f = 0.13) showed no starting material. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate = 10/1 to 0:1 and 0.5% TEA). Afforded compound **11** (7.50 g, 12.4 mmol, 59.4% yield) as yellow oil.

[0356] ¹H NMR: ET32436-28-P1A (400 MHz, DMSO-*d*₆)

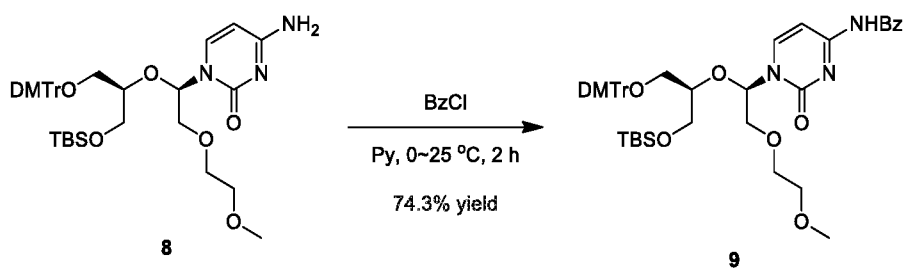
[0357] δ 11.39 (s, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.32-7.28 (m, 4H), 7.20-7.16 (m, 5H), 6.87-6.85 (m, 4H), 5.98 (t, *J* = 6.0 Hz, 1H), 5.54 (d, *J* = 4.0 Hz, 1H), 4.74 (t, *J* = 5.2 Hz, 1H), 3.73 (s, 7H), 3.71 (s, 1H), 3.63-3.59 (m, 1), 3.51-3.55 (m, 2), 3.42-3.41 (m, 4H), 3.21 (s, 3H), 3.00-2.94 (m, 2H).

Example 16. General procedure for preparation of U-UNA-2'MOE 3'Phosphoramidite



[0358] To a solution of compound **11** (8.0 g, 13.2 mmol, 1.00 *eq*) in DCM (48.0 mL) was added 1H-imidazole-4,5-dicarbonitrile (2.34 g, 19.8 mmol, 1.50 *eq*) at 0 °C, and then compound

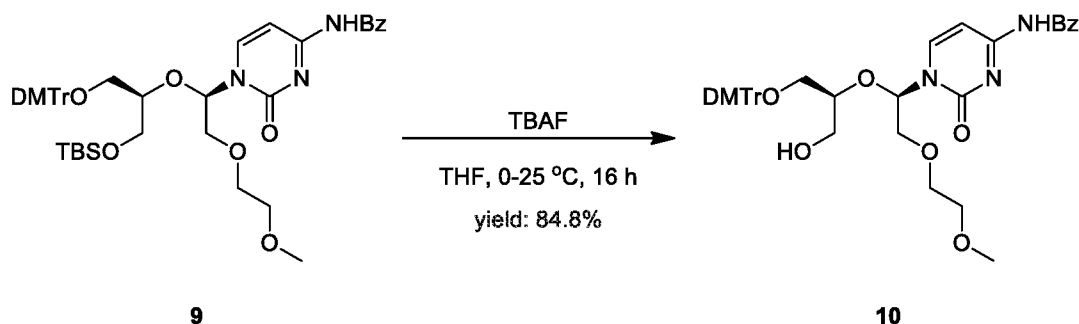
[0362] MS: M-1 = 805.3



[0364] To a solution of compound **8** (40 g, 55.6 mmol, 1.00 *eq*) in Py (240 mL), was added BzCl (11.7 g, 83.3 mmol, 9.68 mL, 1.50 *eq*) at 0 °C, the reaction mixture was stirred 25 °C for 2 h. TLC (Dichloromethane: Methanol = 10: 1, R_f = 0.43) showed no starting material. The mixture was diluted with aq. NaHCO₃ (500 mL) and extracted with DCM (500 mL x 2), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. Concentrate the combined organic layer to give the crude product. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate = 10/1 to 0/1 and 0.5% TEA). Afforded compound **9** (34.0 g, 41.3 mmol, 74.3% yield) as yellow oil

[0365] ¹H NMR: (400 MHz, DMSO-*d*₆) δ 11.36 (s, 1H), 8.58-8.57 (m, 1H), 8.20-8.18 (m, 1H), 8.04-8.02 (m, 2H), 7.64-7.51 (m, 3H), 7.38-7.15 (m, 12H), 6.86-6.83 (m, 4H), 6.16 (t, J = 5.2 Hz, 1H), 3.73-3.69 (m, 4H), 3.67 (s, 6H), 3.60-3.52 (m, 4H), 3.42-3.39 (m, 2H), 3.21-3.19 (m, 3H), 3.00-2.99 (m, 2H), 0.77 (s, 9H), -0.03 (d, J = 8.0 Hz, 6H).

Example 18. General procedure for preparation of compound 10

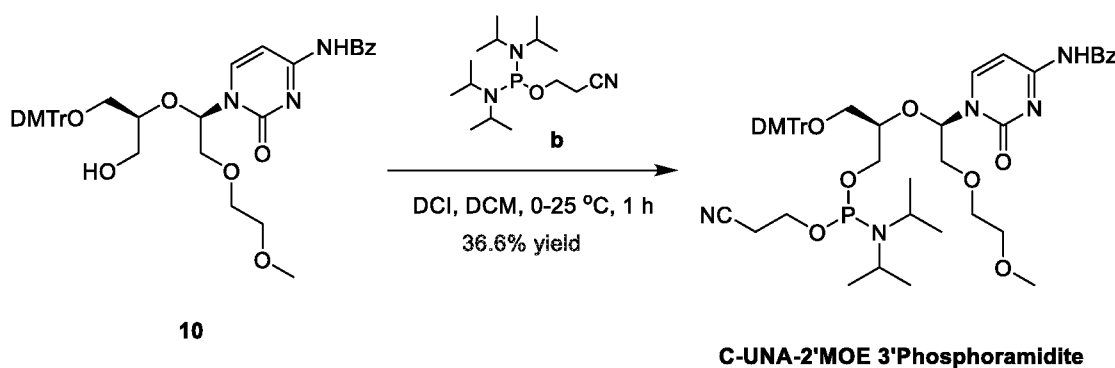


[0366] To a solution of compound **9** (30 g, 36.4 mmol, 1.00 *eq*) in THF (300 mL), was added TBAF (1 M, 40.1 mL, 1.10 *eq*) at 0 °C, the reaction mixture was stirred at 25 °C for 16 h. TLC (Dichloromethane: Methanol = 10: 1, R_f = 0.24) showed no starting material. The mixture was diluted with aq. NaHCO₃ (600 mL) and extracted with DCM (500 mL x 2), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. Concentrate the combined organic layer to give the crude product. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate = 10/1 to 0:1 and 0.5% TEA). Afforded compound **10** (16.5 g, 23.3 mmol, 63.9% yield) as yellow oil.

[0367] ¹H NMR: (400 MHz, DMSO-*d*₆) δ 11.36 (s, 1H), 8.20 (d, J = 7.2 Hz, 1H), 8.03 (d, J = 7.6 Hz, 2H), 7.64 (t, J = 7.4 Hz, 1H), 7.55-7.51 (m, 2H), 7.37 (d, J = 7.2 Hz, 1H), 7.31-7.29 (m,

4H), 7.17 (d, $J=8.8$ Hz, 5H), 6.86 (d, $J=8.8$ Hz, 4H), 6.17 (t, $J=4.8$ Hz, 1H), 4.77 (t, $J=5.4$ Hz, 1H), 3.76-3.74 (m, 2H), 3.68 (s, 7H), 3.60-3.58 (m, 2H), 3.46-3.39 (m, 4H), 3.20 (s, 3H), 3.05-2.96 (m, 2H).

Example 19. General procedure for preparation of C-UNA-2'MOE 3'Phosphoramidite



[0368] To a solution of compound **10** (13.3 g, 18.7 mmol, 1.00 *eq*) in DCM (130 mL) was added 1H-imidazole-4,5-dicarbonitrile (3.32 g, 28.1 mmol, 1.50 *eq*) at 0 °C, and then compound **b** (9.60 g, 31.9 mmol, 10.1 mL, 1.70 *eq*) was added. The reaction mixture was stirred at 25 °C for 1 h. TLC (Petroleum ether: Ethyl acetate = 1:1, $R_f = 0.43$) showed no starting material. The reaction mixture was purified by column chromatography (SiO₂, **Petroleum ether/Ethyl acetate = 1/1-0/1, 5% TEA**). Afforded compound **C-UNA-2'MOE 3'Phosphoramidite** as white solid.

[0369] ¹H NMR: (400 MHz, DMSO-*d*₆)

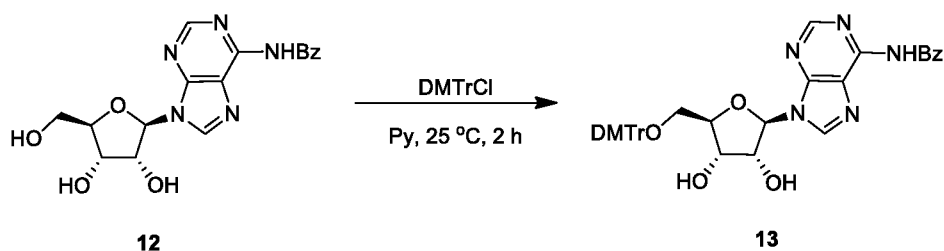
[0370] δ 11.37 (s, 1H), 8.19 (d, $J=6.8$ Hz, 1H), 8.03 (d, $J=7.6$ Hz, 2H), 7.64 (t, $J=7.4$ Hz, 1H), 7.53 (t, $J=7.6$ Hz, 2H), 7.36 (d, $J=7.2$ Hz, 1H), 7.29-7.26 (m, 4H), 7.18-7.15 (m, 5H), 6.85 (d, $J=8.4$ Hz, 4H), 6.15 (s, 1H), 3.87-3.68 (m, 11H), 3.64-3.57 (m, 4H), 3.54-3.44 (m, 2H), 3.40 (s, 2H), 3.20 (s, 3H), 3.12-2.30 (m, 2H), 2.74-2.69 (m, 2H), 1.12-1.10 (m, 6H), 1.05-1.01 (m, 6H)

[0371] ³¹P NMR: (162 MHz, DMSO-*d*₆): δ 147.57

[0372] HPLC: 98.4% purity

[0373] MS: M-1 = 909.3

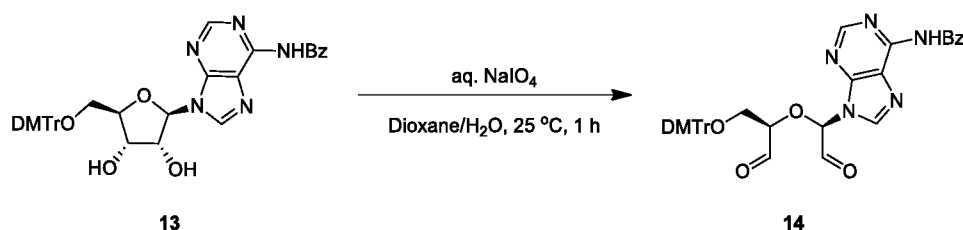
Example 20. General procedure for preparation of compound 13



[0374] To a solution of compound **12** (200 g, 539 mmol, 1.00 *eq*) in Py (2.00 L) was added DMTCl (200 g, 592 mmol, 1.10 *eq*), then stirred at 25 °C for 2 h. TLC (Dichloromethane: Methanol = 20: 1, R_f = 0.44) showed no starting material. The reaction mixture was added MeOH (150 mL), then concentrated in vacuum. The residue was dissolved in EtOAc (2.00 L), washed with aq. acetic acid (500 mL x 2) and brine (500 mL), dried with anhydrous Na_2SO_4 , filtered and concentrated in vacuum. The residue was washed with MTBE (1.00 L x 2). Afforded compound **13** (280 g, crude) as yellow solid.

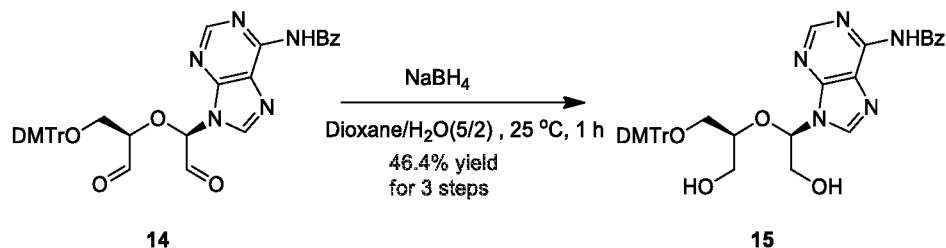
[0375] ^1H NMR: (400 MHz, $\text{DMSO}-d_6$): δ 11.18 (s, 1H), 8.68 (s, 1H), 8.58 (s, 1H), 8.04 (d, J = 7.2 Hz, 1H), 7.65-7.63 (m, 1H), 7.57-7.53 (m, 2H), 7.37-7.36 (m, 2H), 7.20-7.28 (m, 10H), 7.07-7.09 (m, 2H), 6.85-6.82 (m, 6H), 6.07 (d, J = 4.0 Hz, 1H), 5.61 (d, J = 4.0 Hz, 1H), 5.24 (d, J = 4.0 Hz, 1H), 4.79-4.78 (m, 1H), 4.34-4.32 (m, 1H), 4.12-4.11 (m, 1H), 3.74-3.72 (m, 9H), 3.26-3.25 (m, 2H).

Example 21. General procedure for preparation of compound **14**



[0376] To a solution of compound **13** (80.0 g, 119 mmol, 1.00 *eq*) in Dioxane (1.20 L) was added the solution of NaIO_4 (27.9 g, 130 mmol, 7.24 mL, 1.10 *eq*) in H_2O (400 mL) at 25 °C, then stirred at 25 °C for 1 h. TLC (Dichloromethane: Methanol = 15: 1, R_f = 0.32) showed no starting material. The three batches were combined for working up and purification. The reaction mixture was filtered and the filtrate was used for next directly. Afforded compound **14** (240 g, crude) as yellow oil. The crude was used for next directly.

Example 22. General procedure for preparation of compound **15**



[0377] To a solution of compound **14** (80.0 g, 119 mmol, 1.00 *eq*) in dioxane (1.20 L) was added NaBH_4 (4.96 g, 131 mmol, 1.10 *eq*) at 25 °C, then stirred at 25 °C for 1 h. TLC (Dichloromethane: Methanol = 15: 1, R_f = 0.34) showed no starting material. The reaction mixture was added AcOH/Py (1/1, 50.0 mL), then concentrated in vacuum. The three batches

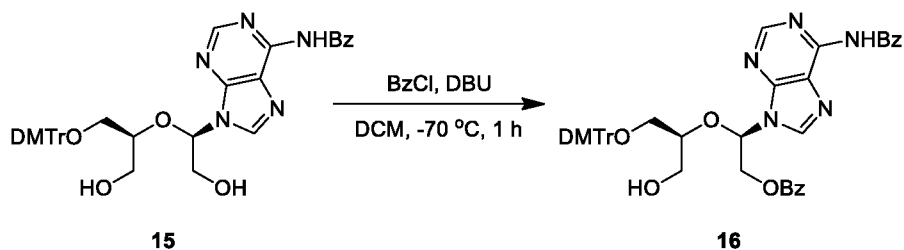
were combined with ET32454-7-P1A for working up and purification. The residue was extracted with EtOAc (1.00 L x 2), washed with aq. NaHCO₃ (600 mL) and brine (600 mL), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by column chromatography (SiO₂, DCM: MeOH = 50:1 to 10:1, 1% TEA). Afforded compound **15** (230.0 g, 46.4% yield for 3 steps) as yellow solid.

[0378] **LCMS:** RT = 2.213 min, M-1 = 674.3.

[0379] **¹H NMR:** (400 MHz, DMSO-*d*₆)

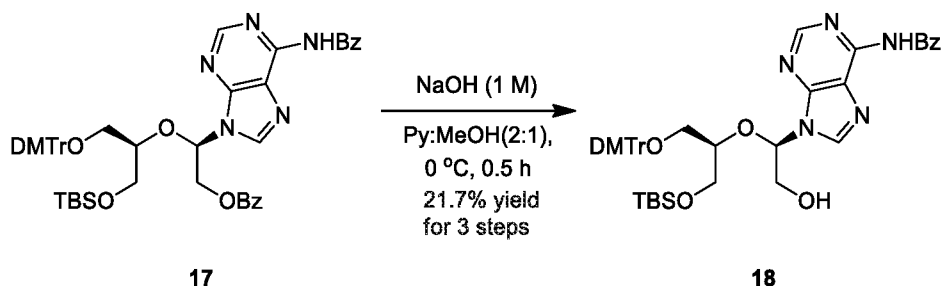
[0380] δ 11.25 (s, 1H), 8.75 (s, 1H), 8.67 (s, 1H), 8.07-8.05 (m, 2H), 7.67-7.63 (m, 1H), 7.57-7.53 (m, 2H), 7.22-7.12 (m, 3H), 7.07-7.05 (m, 1H), 6.97-6.93 (m, 4H), 6.79-6.77 (m, 4H), 6.05 (t, *J* = 12 Hz, 1H), 5.31-5.26 (m, 1H), 4.83 (t, *J* = 8.0 Hz, 1H), 4.14-4.04 (m, 2H), 3.84 (s, 1H), 3.69 (s, 6H), 3.49-3.47 (m, 2H), 2.89-2.78 (m, 1H), 2.77-2.75 (m, 1H).

Example 22. General procedure for preparation of compound 16



starting material. The reaction mixture was washed with aq. NaHCO₃ (800 mL x 2) and brine (800 mL x 2), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. Afforded compound **17** (160 g, crude) as yellow solid. The crude was used for next directly.

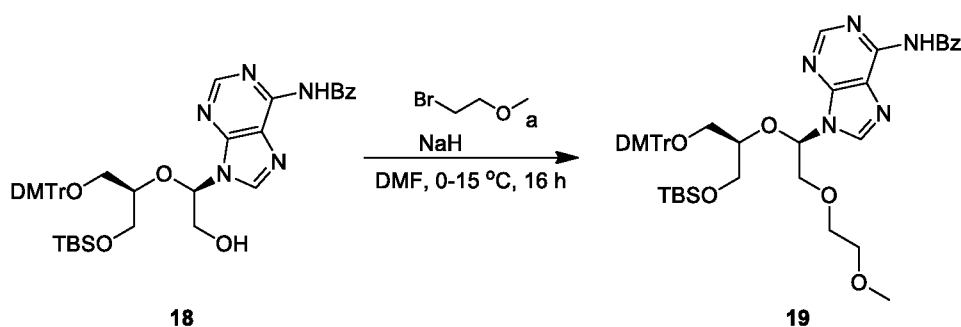
Example 23. General procedure for preparation of compound 18



To a solution of compound of **17** (200 g, 224 mmol, 1.00 *eq*) in Py (1.20 L) and MeOH (480 mL) was added NaOH (1 M, 224 mL, 1.00 *eq*) dropwise at 0 °C, then stirred at 0 °C for 0.5 h. TLC (Dichloromethane: Methanol = 10: 1, R_f = 0.42) showed no starting material. The reaction mixture was extracted with EtOAc (1.00 L x 2), washed with aq.NH₄Cl (1.00 L x 2) and brine (800 mL), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. ET32454-18-P1A was combined with ET32454-19-P1A for purification. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate = 10/1 to DCM: MeOH = 20:1, 1% TEA). Afforded compound **18** (58 g, 21.7% yield for 3 steps) as white solid.

[0383] ¹H NMR: (400 MHz, DMSO-*d*₆) δ 11.24 (s, 1H), 8.75 (s, 1H), 8.70 (s, 1H), 8.07-8.05 (m, 2H), 7.69-7.65 (m, 1H), 7.59-7.56 (m, 2H), 7.24-7.20 (m, 2H), 7.17-7.13 (m, 1H), 7.08-7.06 (m, 1H), 6.99-6.94 (m, 4H), 6.81-6.78 (m, 4H), 6.07-6.04 (m, 1H), 5.30-5.27 (t, *J* = 12 Hz, 1H), 4.20-4.14 (m, 1H), 4.06-3.99 (m, 1H), 3.88-3.85 (m, 1H), 3.76-3.70 (m, 7H), 3.61-3.57 (m, 1H), 0.80 (s, 9H), 0.03 (d, *J* = 12 Hz, 6H).

[0384] Example 24. General procedure for preparation of compound 19

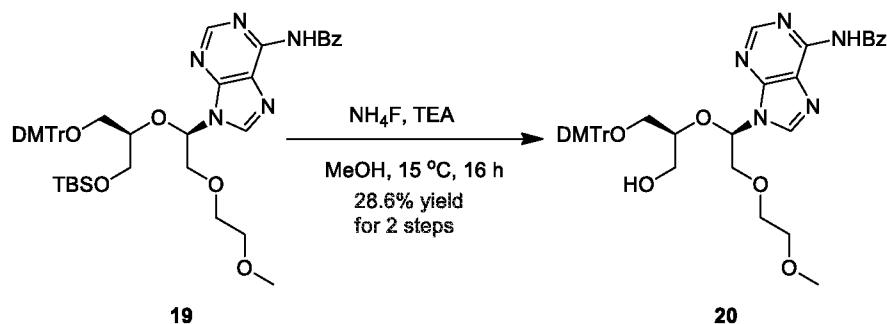


To a solution of NaH (9.52 g, 238 mmol, 60% purity, 3.30 *eq*) in DMF (300 mL) was added the solution of compound **18** (57.0 g, 72.2 mmol, 1.00 *eq*) dropwise at 0 °C, then stirred at 0 °C for 1 h. The mixture was added compound **a** (15.0 g, 108 mmol, 10.2 mL, 1.50 *eq*) dropwise at 0

°C, and then stirred at 15 °C for 15 h. TLC (Dichloromethane: Methanol = 10: 1, R_f = 0.43) showed no starting material. The reaction mixture was poured into aq. NH_4Cl (2.00 L), extracted with EtOAc (800 mL x 2), washed with brine (500 mL x 2), dried with anhydrous Na_2SO_4 , filtered and concentrated in vacuum. Afforded compound **19** (60.0 g, crude) as yellow oil. The crude was used for next directly.

[0385] LCMS: RT = 3.219 min, MS-1 = 846.4.

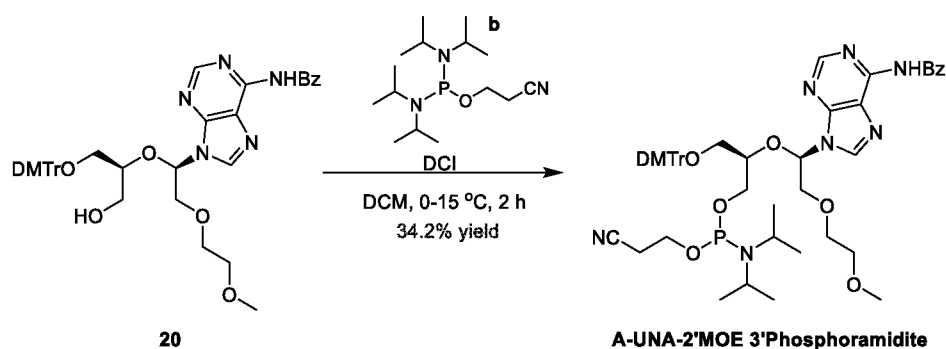
Example 25. General procedure for preparation of compound 20



[0386] To a solution of compound **19** (60.0 g, 70.8 mmol, 1.00 *eq*) in MeOH (360 mL) was added NH_4F (21.0 g, 566 mmol, 8.00 *eq*) and TEA (14.3 g, 142 mmol, 19.7 mL, 2.00 *eq*) at 15 °C, then stirred at 15 °C for 16 h. TLC (Dichloromethane: Methanol = 10: 1, R_f = 0.33) showed no starting material. The reaction mixture was concentrated in vacuum. The residue was purified by column chromatography (SiO_2 , DCM: MeOH = 100:1 to 10:1, 1% TEA). Afforded compound **20** (16.5 g, 22.5 mmol, 28.6% yield) as white solid.

[0387] ^1H NMR: (400 MHz, $\text{DMSO}-d_6$) δ 11.24 (s, 1H), 8.76 (s, 1H), 8.71 (s, 1H), 8.07-8.05 (m, 2H), 7.69-7.65 (m, 1H), 7.59-7.56 (m, 2H), 7.24-7.20 (m, 2H), 7.17-7.13 (m, 1H), 7.08-7.06 (m, 1H), 6.99-6.94 (m, 4H), 6.81-6.78 (m, 4H), 6.25-6.22 (m, 1H), 4.84-4.82 (t, J = 6 Hz, 1H), 4.30-4.25 (m, 1H), 4.09-4.05 (m, 1H), 3.87-3.85 (m, 1H), 3.69 (d, J = 2 Hz, 7H), 3.62-3.60 (m, 2H), 3.50-3.47 (m, 2H), 3.39-3.37 (m, 2H), 3.17 (s, 3H), 2.90-2.86 (m, 1H), 2.77-2.73 (m, 1H).

Example 26. General procedure for preparation of A-UNA-2'MOE 3'Phosphoramidite



[0388] To a solution of compound **20** (15.0 g, 20.4 mmol, 1.00 *eq*) in dry DCM (90.0 mL) was added **DCI** (3.62 g, 30.7 mmol, 1.50 *eq*) and compound **b** (10.5 g, 34.8 mmol, 11.0 mL, 1.70 *eq*) at 0 °C, then stirred at 15 °C for 2 h. TLC (Dichloromethane: Acetone = 3:1, R_f = 0.53) showed no starting material. The solution was poured into the silica gel column directly. The reaction mixture was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate = 5/1 to 0/1, 1% TEA). Afforded **A-UNA-2'MOE 3'Phosphoramidite** (7.00 g, 34.2% yield) as white solid.

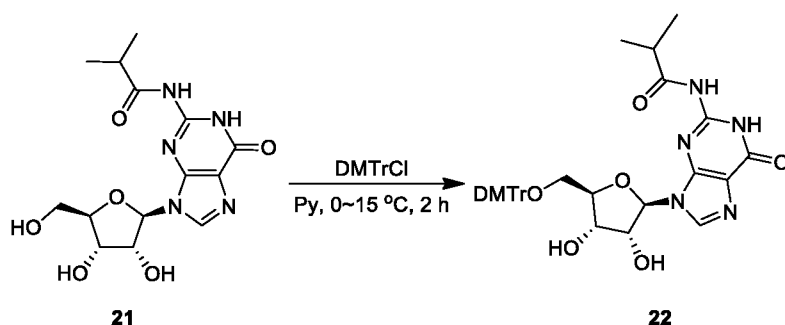
[0389] ¹H NMR: (400 MHz, DMSO-*d*₆) δ 11.26 (s, 1H), 8.77-8.72 (m, 2H), 8.06 (d, J = 7.6 Hz, 2H), 7.63 (t, J = 14.8 Hz, 1H), 7.56 (t, J = 15.2 Hz, 2H), 7.20-7.12 (m, 3H), 7.08-7.04 (m, 2H), 6.98-6.92 (m, 4H), 6.79-6.76 (m, 4H), 6.22 (t, J = 12 Hz, 1H), 4.27-4.24 (m, 1H), 4.04-3.98 (m, 2H), 3.69-3.66 (m, 10H), 3.63-3.62 (m, 2H), 3.62-3.60 (m, 2H), 3.39-3.37 (m, 2H), 3.17 (s, 3H), 2.96-2.71 (m, 4H), 1.19-1.03 (m, 12H).

³¹P NMR: (162 MHz, DMSO-*d*₆) δ 147.62, 147.46

HPLC: 97.8% purity

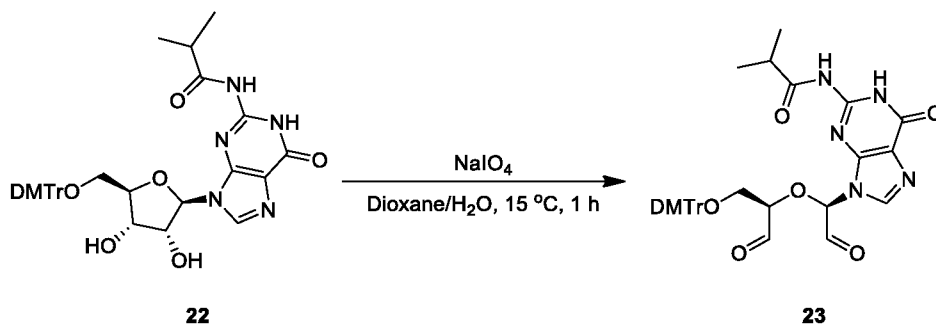
[0390] MS: M-1 = 932.2

Example 27. General procedure for preparation of compound **22**

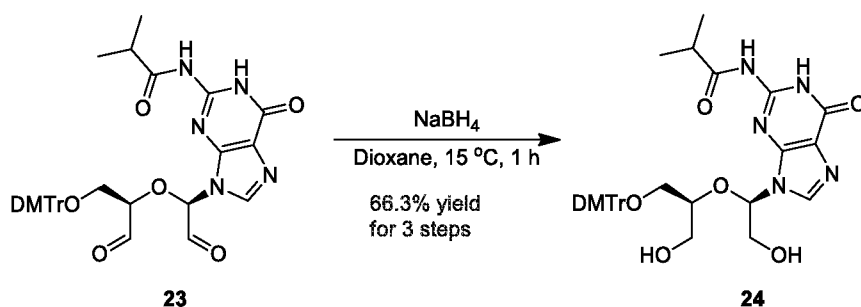


[0391] To a solution of compound **21** (150.0 g, 424.5 mmol, 1.00 *eq*) in Py (900 mL) was added DMTCl (158.2 g, 467.0 mmol, 1.10 *eq*) at 0 °C, then stirred at 15 °C for 2 h. TLC (Dichloromethane: Methanol = 20: 1, R_f = 0.38) showed no starting material. The reaction mixture was added MeOH (150 mL), then concentrated in vacuum. The residue was dissolved in EtOAc (1000 mL), washed with aq. acetic acid (500 mL x 2) and brine (500 mL), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was washed with MTBE (1000 mL x 2). Afforded compound **22** (284.0 g, crude) as yellow solid.

[0392] ¹H NMR: (400 MHz, DMSO-*d*₆) δ 12.10 (s, 1H), 11.66 (s, 1H), 8.58 (d, J = 4.0 Hz, 1H), 8.12 (s, 1H), 7.38-7.35 (m, 3H), 7.33-7.25 (m, 8H), 7.23-7.20 (m, 1H), 6.84-6.80 (m, 4H), 5.86 (d, J = 4.8 Hz, 1H), 5.62 (d, J = 5.6 Hz, 1H), 5.19 (d, J = 5.6 Hz, 1H), 4.54-4.53 (m, 1H), 4.22-4.20 (m, 1H), 3.74 - 3.72 (m, 7H), 3.25-3.18 (m, 1H), 2.78-2.76 (m, 1H), 2.76-2.75 (m, 1H), 1.13 - 1.10 (m, 6H).

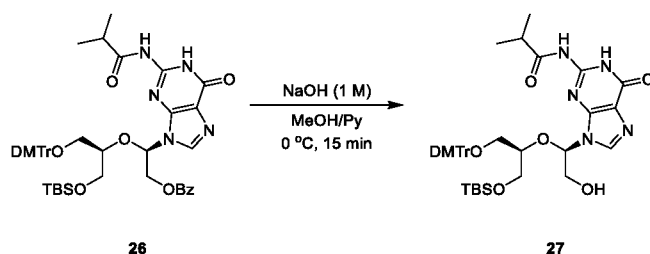
Example 28. General procedure for preparation of compound 23

[0393] Three batches were carried in parallel. To a solution of compound **22** (80.0 g, 122.0 mmol, 1.00 *eq*) in dioxane (1600 mL) was added the solution of NaIO₄ (26.1 g, 122.0 mmol, 6.76 mL, 1.00 *eq*) in H₂O (640 mL) at 15 °C, then stirred at 15 °C for 1 h. TLC (Dichloromethane: Methanol = 15: 1, R_f = 0.28) showed no starting material. The reaction mixture was filtered and the filtrate was used for next directly. Afforded compound **23** (239.3 g, crude) as yellow oil. The crude was used for next directly.

Example 29. General procedure for preparation of compound 24

[0394] To a solution of compound **23** (79.8 g, 122.0 mmol, 1.00 *eq*) in dioxane (1400 mL) was added NaBH₄ (4.62 g, 122.0 mmol, 1.00 *eq*) at 15 °C, then stirred at 15 °C for 1 h. TLC (Dichloromethane: Methanol = 15: 1, R_f = 0.29) showed no starting material. The reaction mixture was added AcOH/Py (1/1, 40 mL), then concentrated in vacuum. The three batches were combined for working up and purification. The residue was extracted with EtOAc (1000 mL x 2), washed with aq. NaHCO₃ (600 mL) and brine (600 mL), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by column chromatography (SiO₂, DCM: MeOH = 50:1 to 10:1, 1% TEA). Afforded compound **24** (206.0 g, 66.3% yield for 3 steps) as yellow solid.

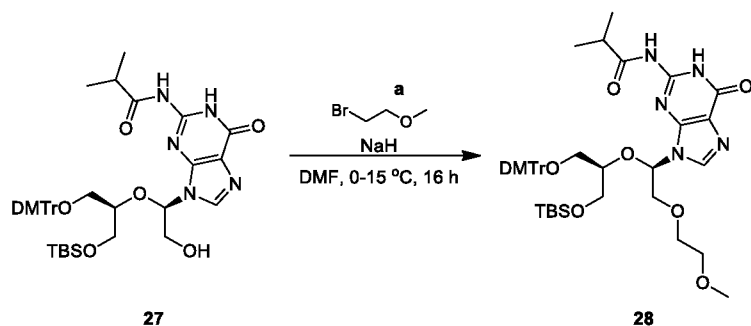
[0395] ¹H NMR: (400 MHz, DMSO-*d*₆) δ 12.11 (s, 1H), 11.71 (s, 1H), 8.17 (s, 1H), 7.21-7.19 (m, 2H), 7.10-7.01 (m, 3H), 6.98-6.96 (m, 4H), 6.80-6.77 (m, 4H), 5.71 (t, *J* = 6.4 Hz, 1H), 5.21 (t, *J* = 5.6 Hz, 1H), 4.78 (t, *J* = 5.2 Hz, 1H), 3.98-3.97 (m, 2H), 3.97-3.76 (m, 1H), 3.71 (s, 6H), 3.44-3.42 (m, 2H), 2.86-2.75 (m, 3H), 1.12-1.06 (m, 6H).



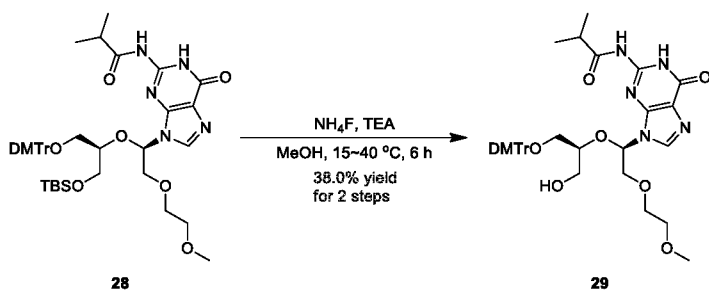
[0398] To a solution of compound of **26** (240.0 g, 274 mmol, 1.00 *eq*) in Py (1200 mL) and MeOH (480 mL) was added NaOH (1 M, 313 mL, 1.14 *eq*) dropwise at 0 °C, then stirred at 0 °C for 15 min. TLC (Dichloromethane: Methanol = 20: 1, R_f = 0.21) showed no starting material. The reaction mixture was extracted with EtOAc (1000 mL x 2), washed with aq.NH₄Cl (1000 mL x 2) and brine (1000 mL), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate = 10/1 to DCM: MeOH = 20:1, 1% TEA). Afforded compound **27** (50 g, 21.6% yield) as white solid.

[0399] ¹H NMR: (400 MHz, DMSO-*d*₆) δ 12.09 (br s, 1H), 11.64 (br s, 1H), 8.20 (s, 1H), 7.21-7.10 (m, 2H), 7.10-7.01 (m, 3H), 6.99-6.95 (m, 4H), 6.80-6.76 (m, 4H), 5.64 (t, J = 6.4 Hz, 1H), 5.16 (t, J = 6.0 Hz, 1H), 4.07-4.05 (m, 1H), 3.93-3.92 (m, 1H), 3.71-3.51 (m, 8H), 2.85-2.82 (m, 1H), 2.80-2.50 (m, 3H), 1.10 (t, J = 6.0 Hz, 6H), 0.74 (s, 9H), -0.06 (d, J = 13.6 Hz, 6H).

Example 32. General procedure for preparation of compound 28

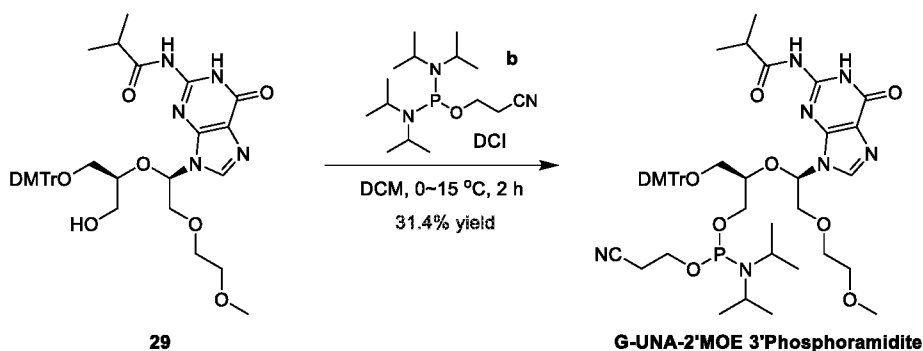


[0400] To a solution of NaH (7.52 g, 188.1 mmol, 60% purity, 3.30 *eq*) in DMF (132 mL) was added the solution of compound **27** (44.0 g, 57.0 mmol, 1.00 *eq*) in DMF (132 mL) dropwise at 0 °C, then stirred at 0 °C for 1 h. The mixture was added compound **a** (11.9 g, 85.5 mmol, 8.03 mL, 1.50 *eq*) dropwise at 0 °C, and then stirred at 15 °C for 15 h. TLC (Dichloromethane: Methanol = 10: 1, R_f = 0.34) showed no starting material. The reaction mixture was poured into aq.NH₄Cl (1500 mL), extracted with EtOAc (500 mL x 2), washed with brine (500 mL), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. Afforded compound **28** (47.3 g, crude) as yellow oil. The crude was used for next directly.

Example 33. General procedure for preparation of compound 29

[0401] To a solution of compound **28** (47.3 g, 57.0 mmol, 1.00 *eq*) in MeOH (280 mL) was added NH_4F (16.9 g, 456.0 mmol, 8.00 *eq*) and TEA (8.00 g, 79.1 mmol, 11.00 mL, 1.39 *eq*) at 15 °C, then stirred at 40 °C for 6 h. TLC (Dichloromethane: Methanol = 10: 1, R_f = 0.33) showed no starting material. The reaction mixture was concentrated in vacuum. The residue was purified by column chromatography (SiO_2 , DCM: MeOH = 100:1 to 10:1, 1% TEA). Afforded compound **29** (15.5 g, 21.6 mmol, 38.0% yield) as white solid.

[0402] ^1H NMR: (400 MHz, $\text{DMSO}-d_6$) δ 12.11 (s, 1H), 11.73 (s, 1H), 8.24 - 8.23 (m, 1H), 7.22 - 7.01 (m, 5H), 6.99 – 6.96 (m, 4H), 6.80 - 6.77 (m, 4H), 5.91 - 5.85 (m, 1H), 4.79 (t, J = 2.6 Hz, 1H), 4.13 - 4.04 (m, 1H), 4.02 – 4.00 (m, 2H), 3.73-3.71 (m, 7H), 3.57-3.56 (m, 2H), 3.45-3.43 (m, 2H), 3.38-3.37 (m, 2H), 3.17 (s, 3H), 2.81-2.77 (m, 3H), 1.14-1.10 (m, 6H).

Example 34. General procedure for preparation of G-UNA-2'MOE 3'Phosphoramidite

[0403] To a solution of compound **29** (15.0 g, 21.0 mmol, 1.00 *eq*) in DCM (90 mL) was added **DCI** (3.71 g, 31.4 mmol, 1.50 *eq*) and compound **b** (10.7 g, 35.6 mmol, 11.31 mL, 1.70 *eq*) at 0 °C, then stirred at 15 °C for 2 h. TLC (Dichloromethane: Acetone = 2:1, R_f = 0.53) showed no starting material. The solution was poured into the silica gel column directly. The reaction mixture was purified by column chromatography (SiO_2 , Petroleum ether/Ethyl acetate = 5/1 to 0/1, 1% TEA). ET32432-31-P1A was combined with ET32432-34-P1A for analysis and delivery. Afforded **G-UNA-2'MOE 3'Phosphoramidite** (6.5 g, 31.4% yield) as yellow solid.

[0404] ^1H NMR: (400 MHz, $\text{DMSO}-d_6$) δ 12.09 (s, 1H), 11.66 (s, 1H), 8.22 (s, 1H), 7.21-6.99 (m, 5H), 6.99-6.98 (m, 4H), 6.80-6.76 (m, 4H), 5.83 (t, J = 6.0 Hz, 1H), 4.16-4.15 (m, 1H),

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3.89-3.71 (m, 1H), 3.66-3.65 (m, 1H), 3.58-3.56 (m, 12H), 3.39-3.38 (m, 2H), 3.37-3.17 (m, 2H), 3.17 (s, 3H), 2.82-2.72 (m, 3H), 2.72-2.70 (m, 2H), 1.12-1.09 (m, 12H), 1.02-0.99 (m, 6H).

[0405] ³¹P NMR: (162 MHz, DMSO-*d*₆) δ 147.46

[0406] HPLC: 95.8% purity

[0407] MS: M-1 = 914.2

[0408] Example 35. Exemplary MSTN siRNA guide strands and passenger strands

comprising UNASense strand and antisense strand sequences targeting MSTN mRNA were generated to include one or more UNAs and/or one or more modified internucleotide linkages. siRNAs comprising the UNA-modified sense strand and/or antisense strands were tested for its stability (T_m) and the efficacy of the siRNA in two different doses (1nM and 0.1 nM, respectively). The sequences and test results are shown in Tables 5 and 6.

TABLE 5

SEQ ID NO	GS (guide, antisense)	SEQ ID NO	PS (passenger, sense)	T _m	1 nM (% mRNA)	0.1 nM (% mRNA)
14239	vpUqsUfsauuAfuuguucUfuUfgc csusu	14240	gsgscaaaaGfAfAfcaaaausa sa	64.57	21.8	50.4
14241	vpUqsUfsauuAfuuguucUfuUfgc csusu	14242	GqGqcaaaaGfAfAfcaaaaau AqAq	55.4	17.3	37.3
14243	vpUqsUfsauuAfuuguucUfuUfgc csusu	14244	GqsGqcaaaaGfAfAfcaaaaau AqsAq	54.56	19.9	45.9
14245	vpUqsUfsauuAfuuguucUfuUfgc csusu	14246	gsgscaaaaGfAfAfcaaaaauA qAq	66.05	20.6	45.6
14247	vpUqsUfsauuAfuuguucUfuUfgc csusu	14248	GqGqcaaaaGfAfAfcaaaaaua sa	57.64	21.6	43.1
14249	vpUqsUfsauuAfuuguucUfuUfgc csusu	14250	GqGqCqaaaGfAfAfcaaaaaua Uqaq	46.72	21.5	52.4
14251	vpUqsUfsauuAfuuguucUfuUfgc csusu	14252	GqGqCqAbaaGfAfAfcaaaaaua AbUqAqAq	51.31	18.7	39.5
14253	vpUqsUfsauuAfuuguucUfuUfgc csusu	14254	GbGbcaaaaGfAfAfcaaaaaua AqAq	74.06	16.1	35.6
14255	vpUqsUfsauuAfuuguucUfuUfgc csusu	14256	GqGqcaaaaGfAfAfcaaaaaua AbAb	62.59	15.8	34.9

vp: vinylphosphate

s: phosphorothioate internucleotide linkage

Xf = 2'-fluoro nucleoside

lower case: 2'-O-methyl

Xb: LNA

TABLE 6

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SEQ ID NO	GS (guide, antisense)	SEQ ID NO	PS (passenger, sense)	Tm	1 nM (% mRNA)	0.1 nM (% mRNA)
14257	vpUqsUfsauuAfuuguucUfuUfgc csusu	14258	gsgscaaaGfAfAfaaaauaasa sa	66.4	24.6	45.4
14259	vpUqsUfsauuAfuuguucUfuUfgc cUqUq	14260	gsgscaaaGfAfAfaaaauaasa sa	66.6	20.6	40.6
14261	vpUqsUfsauuAfuuguucUfuUfG qCqCqsusu	14262	gsgscaaaGfAfAfaaaauaasa sa	51.5	37.3	72.1
14263	vpUqsUfsauuAfuuguucUfuUfG qCqCqTbTb	14264	gsgscaaaGfAfAfaaaauaasa sa	51.5	29.4	61.5
14265	vpUqsUfsauuAfuuguucUfuUfG bCbCbUqUq	14266	gsgscaaaGfAfAfaaaauaasa sa	68.26	49.7	83.7
14267	vpUqsUfsauuAfuuguucUfuUfG qCqCqUqUq	14268	gsgscaaaGfAfAfaaaauaasa sa	52.6	31.7	68.9
14269	vpUqsUfsauuAfuuguucUfuUfgc cUqsUq	14270	gsgscaaaGfAfAfaaaauaasa sa	66.25	16.0	34.8
14271	vpUqsUfsauuAfuuguucUfuUfgc csUqsUq	14272	gsgscaaaGfAfAfaaaauaasa sa	66.35	18.9	41.3
14273	vpUqsUfsauuAfuuguucUfuUfgc csUwsUw	14274	gsgscaaaGfAfAfaaaauaasa sa	64.5	24.9	47.8
14275	vpUqsUfsauuAfuuguucUfuUfgc csUxsUx	14276	gsgscaaaGfAfAfaaaauaasa sa	65.4	21.9	43.7
14277	vpUqsUfsauuAfuuguucUfuUfgc csUysUy	14278	gsgscaaaGfAfAfaaaauaasa sa	64.97	22.5	37.5
14279	vpUqsUfsauuAfuuguucUfuUfgc cUwUw	14280	gsgscaaaGfAfAfaaaauaasa sa	66.55	21.0	36.6
14281	vpUqsUfsauuAfuuguucUfuUfgc cUxUx	14282	gsgscaaaGfAfAfaaaauaasa sa	64.8	15.9	36.0
14283	vpUqsUfsauuAfuuguucUfuUfgc cUyUy	14284	gsgscaaaGfAfAfaaaauaasa sa	66.37	18.8	38.1
14285	vpUqsTbauuAfuuguucUfuUfgcc UqsUq	14286	gsgscaaaGfAfAfaaaauaasa sa	68.36	10.7	34.0
14287	vpUqsTbauuAfuuguucUfuUfgcc UwsUw	14288	gsgscaaaGfAfAfaaaauaasa sa	66.66	14.0	37.2
14289	vpUqsTbauuAfuuguucUfuUfgcc UxsUx	14290	gsgscaaaGfAfAfaaaauaasa sa	67.85	13.5	34.0
14291	vpUqsTbauuAfuuguucUfuUfgcc UysUy	14292	gsgscaaaGfAfAfaaaauaasa sa	68.46	20.6	43.7
14293	vpUqsUqsauuAfuuguucUfuUfg ccsusu	14294	gsgscaaaGfAfAfaaaauaasa sa	65.92	89.8	103.6
14295	vpUqsUfsauuAquuguucUfuUfg ccsusu	14296	gsgscaaaGfAfAfaaaauaasa sa	58.34	63.1	88.8
14297	vpUqsUfsauuAfuuguucUquUfg ccsusu	14298	gsgscaaaGfAfAfaaaauaasa sa	57.26	47.4	88.4
14299	vpUqsUfsauuAfuuguucUfuUqg ccsusu	14300	gsgscaaaGfAfAfaaaauaasa sa	57.45	19.5	48.2
14301	vpUqsUwsauuAfuuguucUfuUfg ccsusu	14302	gsgscaaaGfAfAfaaaauaasa sa	65.7	93.3	85.7
14303	vpUqsUfsauuAfuuguucUwuUfg ccsusu	14304	gsgscaaaGfAfAfaaaauaasa sa		101.6	106.5

1430 5	vpUqsUfsauuAfuuguucUfuUwg ccsusu	143 06	gsgscaaaGfAfAfcaaaaua sa	57.4	24.2	48.4
1430 7	vpUqsUxsauuAfuuguucUfuUfgc csusu	143 08	gsgscaaaGfAfAfcaaaaua sa	69.7	103.4	113.4
1430 9	vpUqsUfsauuAfuuguucUxuUfgc csusu	143 10	gsgscaaaGfAfAfcaaaaua sa	55.3 1	60.2	83.3
1431 1	vpUqsUfsauuAfuuguucUfuUxgc csusu	143 12	gsgscaaaGfAfAfcaaaaua sa	56.8 5	21.9	47.1
1431 3	vpUqsUysauuAfuuguucUfuUfgc csusu	143 14	gsgscaaaGfAfAfcaaaaua sa	68.7	94.8	104.2
1431 5	vpUqsUfsauuAfuuguucUyuUfgc csusu	143 16	gsgscaaaGfAfAfcaaaaua sa	56.5	66.3	92.8
1431 7	vpUqsUfsauuAfuuguucUfuUygc csusu	143 18	gsgscaaaGfAfAfcaaaaua sa	57.3 9	20.7	46.0
1431 9	vpUqsTbsAquuAfuuguucUfuUf gccsusu	143 20	gsgscaaaGfAfAfcaaaaua sa	61.6	69.8	94.0
1432 1	vpUqsUfsauuAbUquuguucUfuUf gccsusu	143 22	gsgscaaaGfAfAfcaaaaua sa	61.7 4	64.6	93.9
1432 3	vpUqsUfsauuAfUquuguucUfuUf gccsusu	143 24	gsgscaaaGfAfAfcaaaaua sa	51	56.2	82.6
1432 5	vpUqsUfsauuAfuuguucUfuTbG qccsusu	143 26	gsgscaaaGfAfAfcaaaaua sa	59.4 2	24.9	57.5
1432 7	vpUqsUfsauuAfuuguucUfuUqTb Gqccsusu	143 28	gsgscaaaGfAfAfcaaaaua sa	50	80.5	93.5
1432 9	vpUqsTbsAquUqUqAbuuguucUf uUfgccsusu	143 30	gsgscaaaGfAfAfcaaaaua sa	66.5	108.2	98.6
1433 1	vpUqsTbsAquUqAbUquuguucUf uUfgccsusu	143 32	gsgscaaaGfAfAfcaaaaua sa	58.9 9	102.0	102.8
1433 3	vpUqsTbsauuAbUquuguucUfuUf gccsusu	143 34	gsgscaaaGfAfAfcaaaaua sa	62.1 6	83.2	95.7
1433 5	vpUqsUfsauuAfuuguucTbuTbG qCqCqsusu	143 36	gsgscaaaGfAfAfcaaaaua sa	55.1 7	54.1	77.4
1433 7	vpUqsUfsauuAfuuguuUqTbUqT bGqccsusu	143 38	gsgscaaaGfAfAfcaaaaua sa	37	82.2	97.0
1433 9	vpUqsUfsauuAquuguucUfuUqg ccsusu	143 40	gsgscaaaGfAfAfcaaaaua sa	44.7	74.8	99.9
1434 1	vpUqsTbsauuAquuguucTbuUqg ccsusu	143 42	gsgscaaaGfAfAfcaaaaua sa	49.9	89.8	89.3
1434 3	vpUqsTbauuAquuguucTbuUqg ccusu	143 44	gsgscaaaGfAfAfcaaaaua sa	52.8	90.6	96.1

vp: vinylphosphate

s: phosphorothioate internucleotide linkage

Xf = 2'-fluoro nucleoside

lower case: 2'-O-methyl

Xb: LNA

Example 36. MSTN siRNA guide strands and passenger strands comprising UNA and their efficacies in downregulating MSTN mRNA *in vitro*

[0409] Four candidate siRNAs targeting MSTN mRNA were tested for its efficacy to downregulate MSTN mRNA *in vitro* in SJCRH30 cells. The sequences of four candidate

siRNAs (R002728-1, R002729-1, R002734-1, R002735-1) and their stability (indicated by T_m) and efficacy (EC₅₀ value) are shown in Table 7. FIG. 3 also shows downregulation of MSTN mRNA in SJCRH30 cells by each candidate siRNA.

TABLE 7

siRNA	SEQ ID NO	PS (passenger, sense)	SEQ ID NO	GS (guide, antisense)	EC ₅₀ (pM)	T _m [C]
R002728-1	14345	aminoC6-gsgscaaaGfAfAfcaaaaua usasa	14346	vpUqsUfsauuAfuuug uucUfuUfgccUqUq	18.41	67.51
R002729-1	14347	aminoC6-gsgscaaaGfAfAfcaaaaua usasa	14348	vpUqsUfsauuAfuuug uucUfuUfgccUqsUq	25.27	67.5
R002734-1	14349	aminoC6-GqGqcaaaGfAfAfcaaaaua auAqAq	14350	vpUqsUfsauuAfuuug uucUfuUfgccsusu	39.45	54
R002735-1	14351	aminoC6-GqsGqcaaaGfAfAfcaaaau aaUAqsAq	14352	vpUqsUfsauuAfuuug uucUfuUfgccsusu	45.19	54.8

vp: vinylphosphate

s: phosphorothioate internucleotide linkage

Xf = 2'-fluoro nucleoside

lower case: 2'-O-methyl

Xb: LNA

Example 37. MSTN siRNA guide strands and passenger strands comprising UNA and their efficacy in downregulating MSTN mRNA *in vivo*

[0410] Four siRNAs targeting MSTN mRNA were tested for its efficacy to downregulate MSTN mRNA *in vivo*. The sequences of four candidate siRNAs (R002728-1, R002729-1, R002734-1, R002735-1) and their stability (indicated by T_m) and efficacy (EC₅₀ value) are shown in Table 7. siRNA targeting MSTN mRNA was conjugated with a-CD71 antibody, and the a-CD71-MSTN siRNA were administered *in vivo* to determine the effect of the a-CD71-MSTN siRNA. Table 8 shows K_d (% mRNA) value and the concentration of siRNAs in the Gastronecmius muscle after administering the siRNA conjugates. FIG. 4 shows a comparison graph of the four MSTN siRNA conjugates.

TABLE 8

siRNA	KD in Gastroc				siRNA in Gastroc			
	[% mRNA]				[nM]			
	d14	d28	d44	d57	d14	d28	d44	d57
R002728-1	27	54	75	92				

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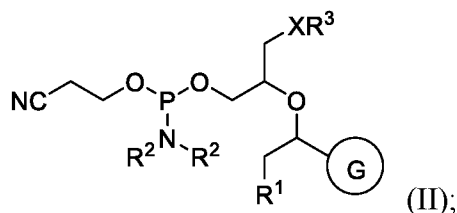
R002729-1	16	22	51	52	19.8	8.0	3.4	2.2
R002734-1	33	44	59	59	8.0	2.4	0.9	0.3
R002735-1	24	18	18	28	14.0	5.1	2.4	0.6

[0411] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

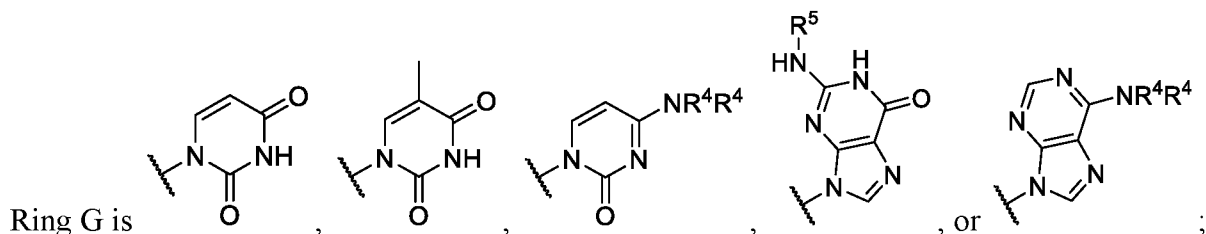
CLAIMS

WHAT IS CLAIMED IS:

1. A compound, or a pharmaceutically acceptable salt or solvate thereof, having the structure of Formula (II):



wherein:



X is O, NH, or C(=O);

R¹ is hydrogen, deuterium, halogen, substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, substituted or unsubstituted C₁-C₁₀ fluoroalkyl, substituted or unsubstituted C₁-C₂₀ heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted -O-aryl, -OR⁶, -C(=O)R⁶, -C(=O)OR⁶, -C(=O)NR⁷R⁷, -OCH₂C(=O)R⁶, -OCH₂C(=O)OR⁶, or -OCH₂C(=O)NR⁷R⁷;

each R² is independently hydrogen, deuterium, or substituted or unsubstituted C₁-C₁₀ alkyl; or two R² are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C₃-C₁₀ heterocycloalkyl;

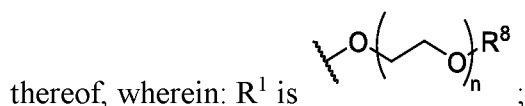
R³ is hydrogen, 4,4'-dimethoxytrityl (DMT), -R⁶, -OR⁶, or -NR⁷R⁷;

each R⁴ is independently hydrogen, substituted or unsubstituted C₁-C₆alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted benzoyl;

R⁵ is -C(=O)R⁶;

each R⁶ and R⁷ are independently hydrogen, substituted or unsubstituted C₁-C₆alkyl, substituted or unsubstituted C₁-C₆fluoroalkyl, substituted or unsubstituted C₃-C₆cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

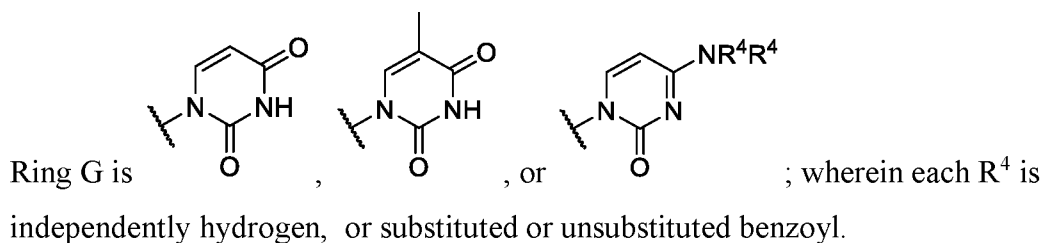
2. The compound of claim 1, or a pharmaceutically acceptable salt or solvate, thereof, wherein: R^1 is halogen, substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_1 - C_{10} fluoroalkyl, substituted or unsubstituted C_1 - C_{20} heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted $-O$ -aryl, $-OH$, $-OR^6$, $-C(=O)R^6$, $-C(=O)OR^6$, $-C(=O)NR^7R^7$, $-OCH_2C(=O)R^6$, or $-OCH_2C(=O)OR^6$, or $-OCH_2C(=O)NR^7R^7$.
3. The compound of claim 1 or 2, or a pharmaceutically acceptable salt or solvate, thereof, wherein: R^1 is halogen, substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_1 - C_{20} heteroalkyl, substituted or unsubstituted $-O$ -aryl, $-OCH_2C(=O)R^6$, or $-OCH_2C(=O)OR^6$, or $-OCH_2C(=O)NR^7R^7$.
4. The compound of any one of claims 1-3, or a pharmaceutically acceptable salt or solvate, thereof, wherein: R^1 is halogen or substituted or unsubstituted C_1 - C_{20} heteroalkyl.
5. The compound of any one of claims 1-4, or a pharmaceutically acceptable salt or solvate,



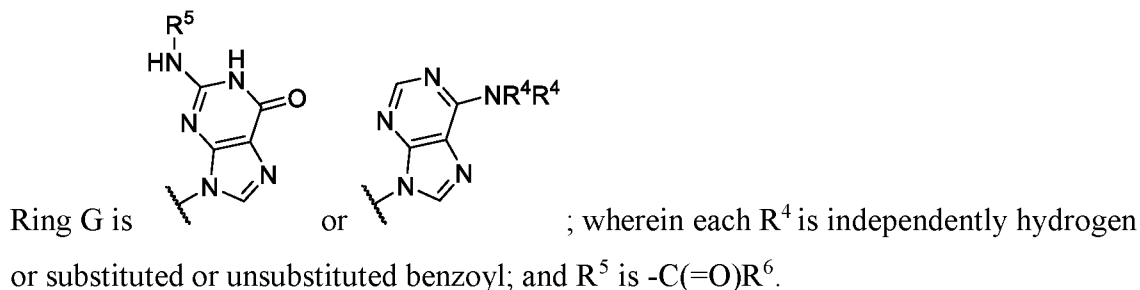
n is 0, 1, 2, 3, 4, or 5; and

R^8 is hydrogen or substituted or unsubstituted C_1 - C_3 alkyl.

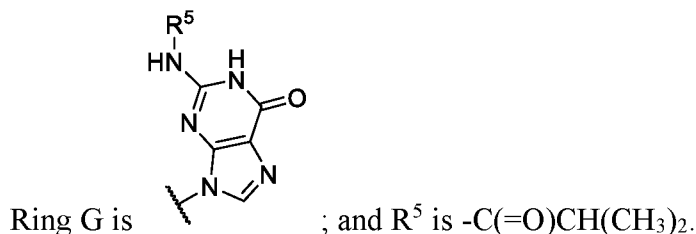
6. The compound of claim 5, or a pharmaceutically acceptable salt or solvate, thereof, wherein: R^8 is substituted or unsubstituted C_1 - C_3 alkyl.
7. The compound of claim 5 or 6, or a pharmaceutically acceptable salt or solvate, thereof, wherein: R^8 is methyl or ethyl.
8. The compound of any one of claims 5-7, or a pharmaceutically acceptable salt or solvate, thereof, wherein n is 1 or 2.
9. The compound of any one of claims 1-3, or a pharmaceutically acceptable salt or solvate, thereof, wherein: R^1 is $-OCH_2C(=O)NR^7R^7$; wherein each R^7 is independently hydrogen or substituted or unsubstituted C_1 - C_3 alkyl.
10. The compound of any one of claims 1-9, or a pharmaceutically acceptable salt or solvate thereof, wherein:



11. The compound of any one of claims 1-9, or a pharmaceutically acceptable salt or solvate thereof, wherein:



12. The compound of any one of claims 1-9 or 11, or a pharmaceutically acceptable salt or solvate thereof, wherein:



13. The compound of any one of claims 1-12, or a pharmaceutically acceptable salt or solvate thereof, wherein each R² is independently hydrogen, deuterium, or substituted or unsubstituted C₁-C₁₀ alkyl.

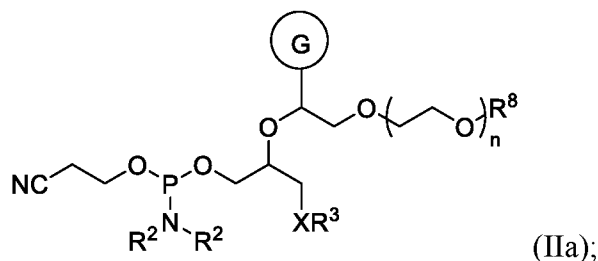
14. The compound of any one of claims 1-13, or a pharmaceutically acceptable salt or solvate thereof, wherein each R² is independently substituted or unsubstituted C₁-C₁₀ alkyl.

15. The compound of any one of claims 1-14, or a pharmaceutically acceptable salt or solvate thereof, wherein each R² is independently isopropyl.

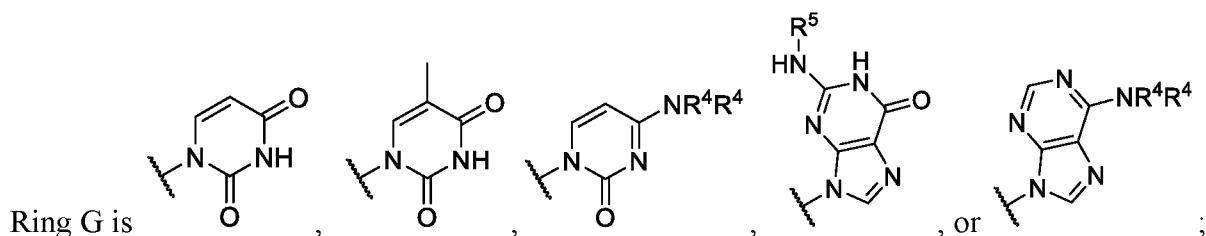
16. The compound of any one of claims 1-12, or a pharmaceutically acceptable salt or solvate thereof, wherein two R² are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C₃-C₁₀ heterocycloalkyl.

17. The compound of any one of claims 1-16, or a pharmaceutically acceptable salt or solvate thereof, wherein X is O; and R³ is 4,4'-dimethoxytrityl (DMT).

18. A compound, or a pharmaceutically acceptable salt or solvate thereof, having the structure of Formula (IIa):



wherein:



X is O, NH, or C(=O);

n is 0, 1, 2, 3, or 4;

R⁸ is substituted or unsubstituted C₁-C₆ alkyl;

each R² is independently hydrogen, deuterium, or substituted or unsubstituted C₁-C₁₀ alkyl;

or two R² are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C₃-C₁₀cycloalkyl;

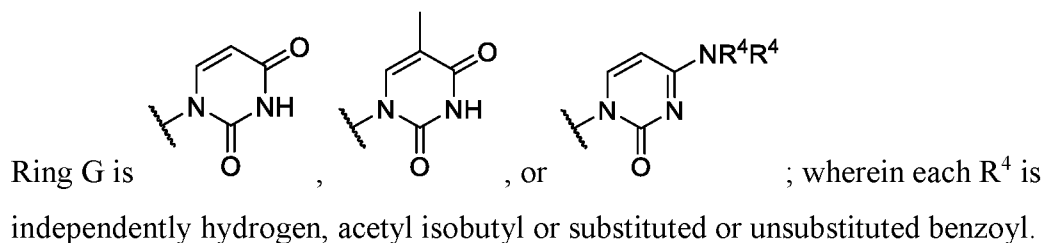
R³ is hydrogen, 4,4'-dimethoxytrityl (DMT), -R⁶, -OR⁶, or -NR⁷R⁷.

each R⁴ is independently hydrogen, substituted or unsubstituted C₁-C₆alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted benzoyl;

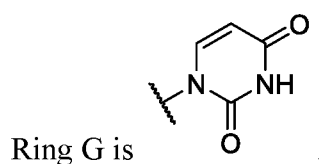
R⁵ is -C(=O)R⁶;

each R⁶ and R⁷ are independently hydrogen, substituted or unsubstituted C₁-C₆alkyl, substituted or unsubstituted C₁-C₆fluoroalkyl, substituted or unsubstituted C₃-C₆cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

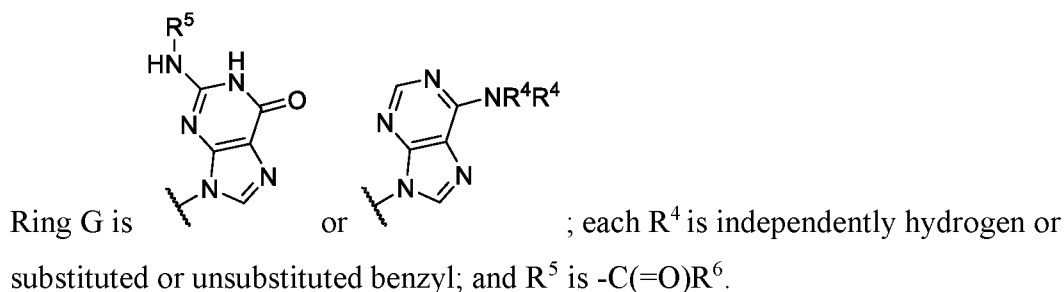
19. The compound of claim **18**, or a pharmaceutically acceptable salt or solvate thereof, wherein:



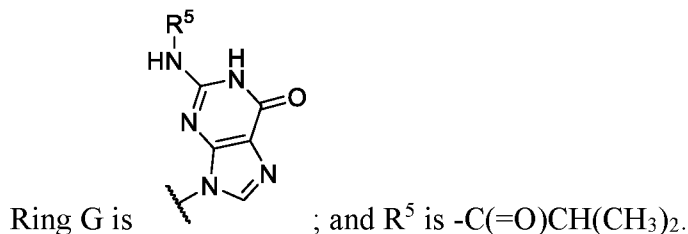
20. The compound of claim **18** or **19**, or a pharmaceutically acceptable salt or solvate thereof, wherein:



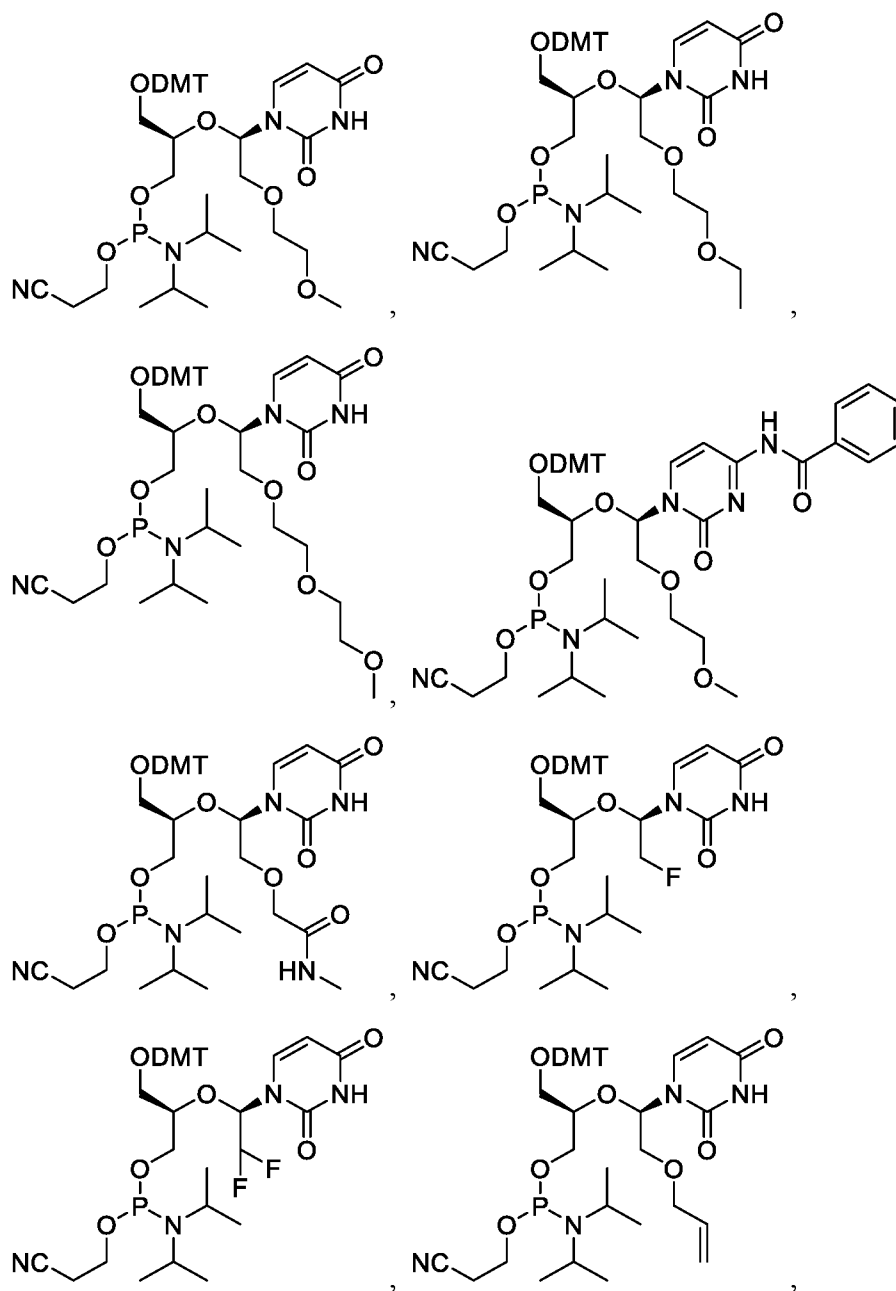
21. The compound of claim **18**, or a pharmaceutically acceptable salt or solvate thereof, wherein:

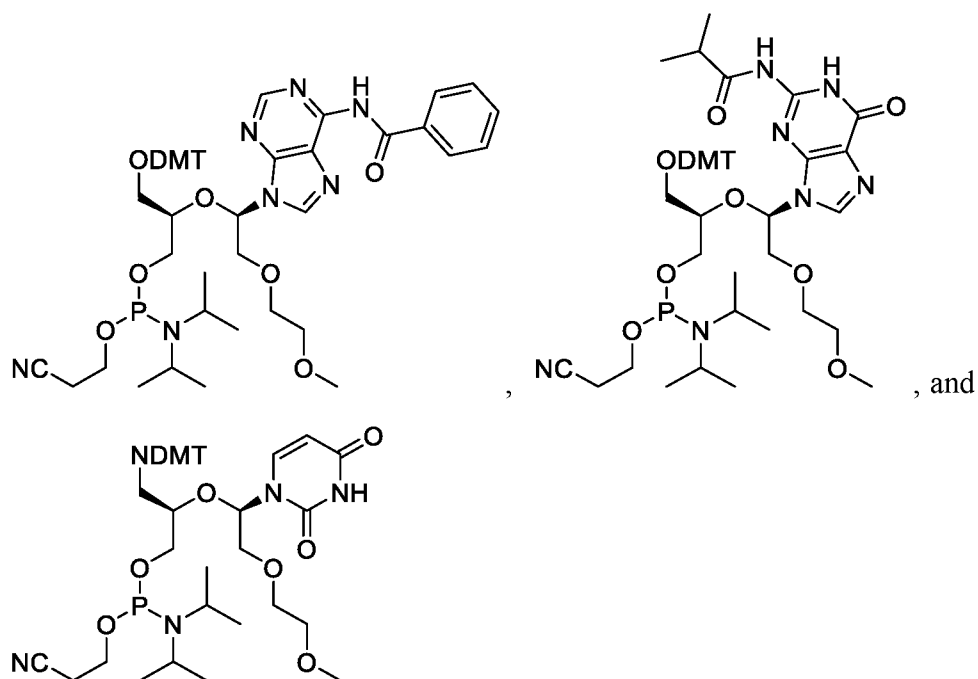


22. The compound of claim **18** or **21**, or a pharmaceutically acceptable salt or solvate thereof, wherein:



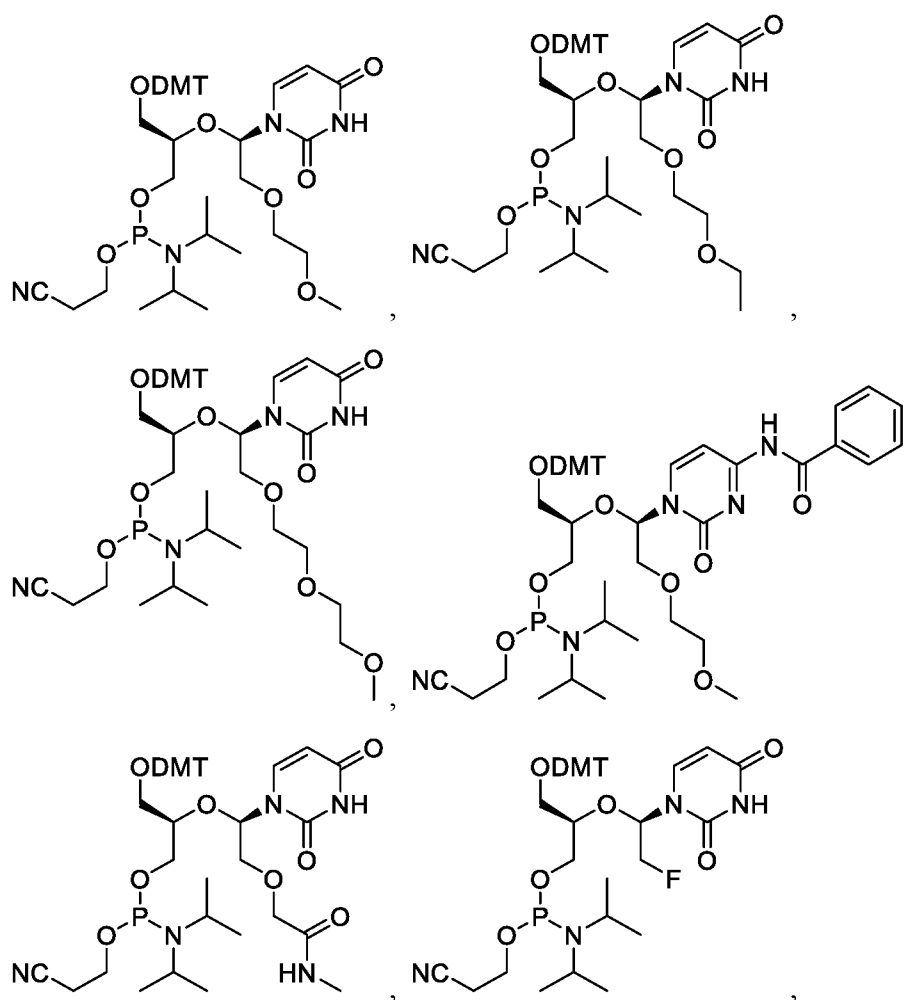
23. The compound of claim **22**, or a pharmaceutically acceptable salt or solvate thereof, wherein; R⁶ is substituted or unsubstituted C₁-C₆ alkyl.
24. The compound of any one of claims **18-23**, or a pharmaceutically acceptable salt or solvate thereof, wherein R⁸ is methyl or ethyl.
25. The compound of any one of claims **18-24**, or a pharmaceutically acceptable salt or solvate thereof, wherein n is 1 or 2.
26. The compound of any one of claims **18-25**, or a pharmaceutically acceptable salt or solvate thereof, wherein n is 1.
27. The compound of any one of claims **18-26**, or a pharmaceutically acceptable salt or solvate thereof, wherein each R² is independently hydrogen, deuterium, or substituted or unsubstituted C₁-C₁₀ alkyl.
28. The compound of any one of claims **18-27**, or a pharmaceutically acceptable salt or solvate thereof, wherein each R² is independently substituted or unsubstituted C₁-C₁₀ alkyl.
29. The compound of any one of claims **18-28**, or a pharmaceutically acceptable salt or solvate thereof, wherein each R² is independently isopropyl.
30. The compound of any one of claims **18-26**, or a pharmaceutically acceptable salt or solvate thereof, wherein two R² are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C₃-C₁₀ heterocycloalkyl.
31. The compound of any one of claims **18-30**, or a pharmaceutically acceptable salt or solvate thereof, wherein R³ is 4,4'-dimethoxytrityl (DMT).
32. The compound of any one of claims **1-31**, or a pharmaceutically acceptable salt or solvate thereof, selected from:

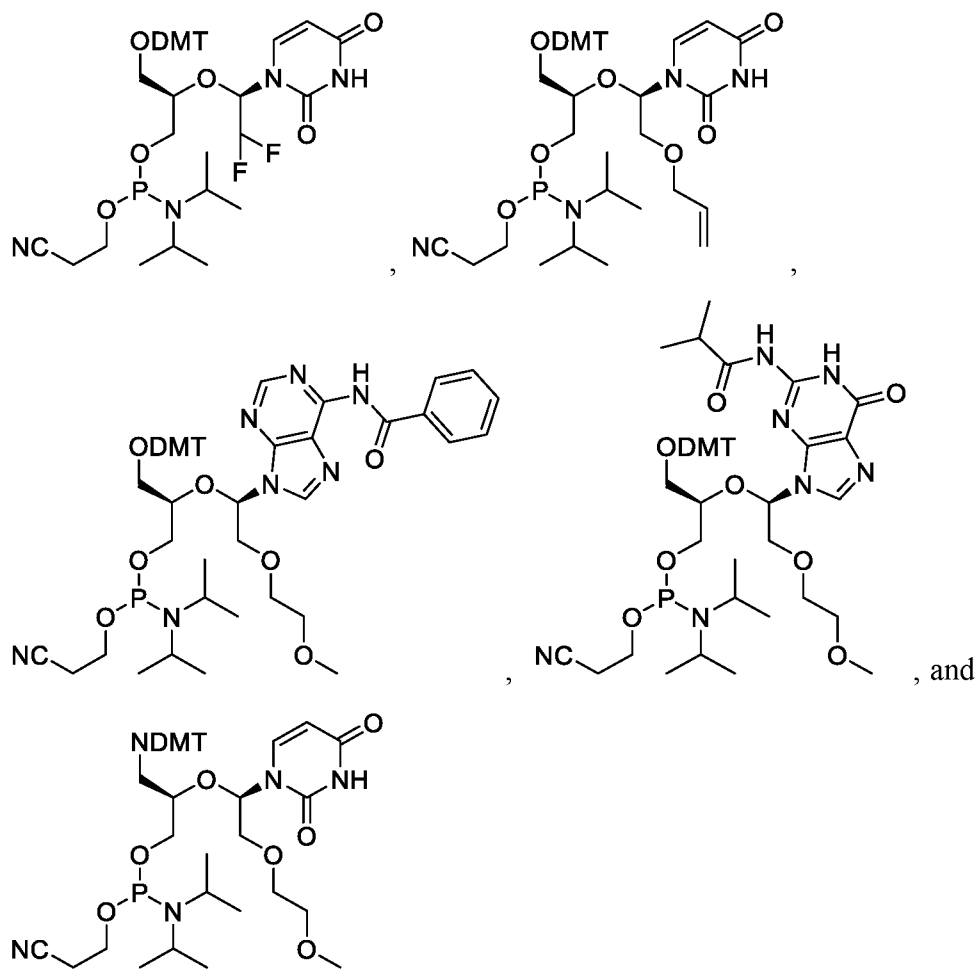




33. The compound of any one of claims 1-32, or a pharmaceutically acceptable salt or solvate thereof, wherein the compound is suitable for the synthesis of oligonucleotides.

34. A compound suitable for the synthesis of oligonucleotides selected from:





35. An oligonucleotide conjugate of Formula (I):

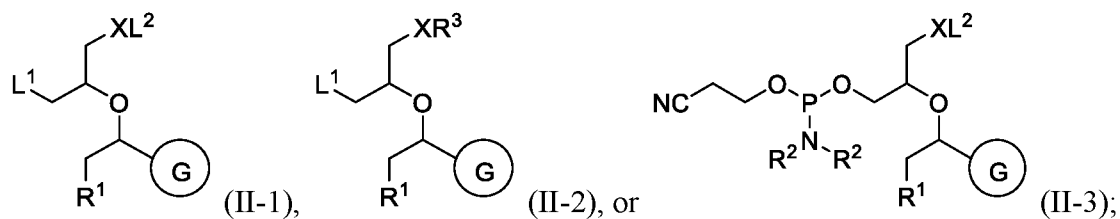
A-B

Formula (I);

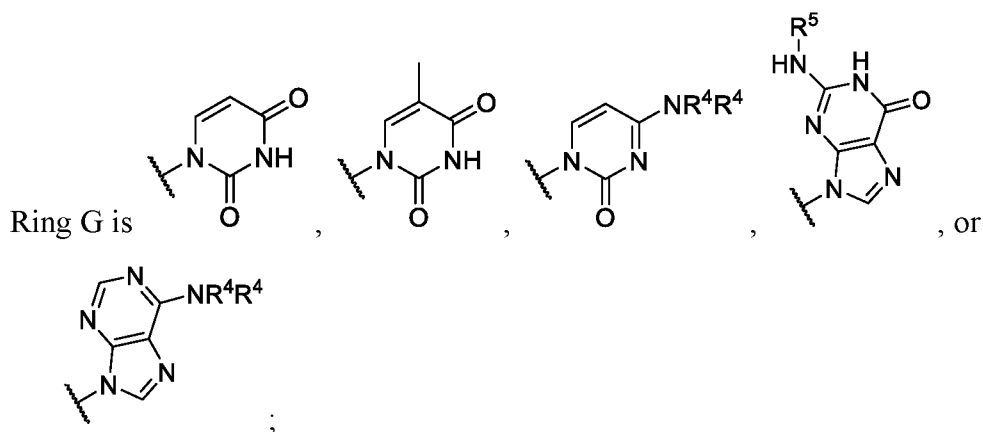
wherein,

A is a binding moiety;

B is an oligonucleotide comprising a compound of, or a compound derived from, Formula (II-1), (II-2), or (II-3); and



wherein:



X is O, NH, or C(=O);

L¹ and L² are each independently absent or a linking moiety selected from a phosphodiester, amide and ester linkage:

R¹ is hydrogen, deuterium, halogen, substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, substituted or unsubstituted C₁-C₁₀ fluoroalkyl, substituted or unsubstituted C₁-C₂₀ heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted -O-aryl, -OR⁶, -C(=O)R⁶, -C(=O)OR⁶, -C(=O)NR⁷R⁷, -OCH₂C(=O)R⁶, -OCH₂C(=O)OR⁶, or -OCH₂C(=O)NR⁷R⁷;

each R² is independently hydrogen, deuterium, or substituted or unsubstituted C₁-C₁₀ alkyl;

or two R² are taken together with the nitrogen atom to which they are attached to form a substituted or unsubstituted C₃-C₁₀ heterocycloalkyl;

R³ is hydrogen, 4,4'-dimethoxytrityl (DMT), -R⁶, -OR⁶, or -NR⁷R⁷;

each R⁴ is independently hydrogen, substituted or unsubstituted C₁-C₆alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted benzoyl;

R⁵ is -C(=O)OR⁶; and

each R⁶ and R⁷ are independently hydrogen, substituted or unsubstituted C₁-C₆alkyl, substituted or unsubstituted C₁-C₆fluoroalkyl, substituted or unsubstituted C₃-C₆cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

36. The oligonucleotide conjugate of claim 35, wherein B is an oligonucleotide comprising a compound of Formula (II-1).

37. The oligonucleotide conjugate of claim 35, wherein B is an oligonucleotide comprising a compound of Formula (II-2).

38. The oligonucleotide conjugate of claim **35**, wherein B is an oligonucleotide comprising a compound derived from Formula (II-3).
39. The oligonucleotide conjugate of any one of claims **35-38**, wherein the oligonucleotide is an RNA oligonucleotide.
40. The oligonucleotide conjugate of any one of the claims **35-39**, comprising at least one modification.
41. The oligonucleotide conjugate of any one of the claims **35-40**, comprising at least one 2' modified nucleotide.
42. The oligonucleotide conjugate of any one of the claims **35-41**, comprising at least one 2' modified nucleotide selected from 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide.
43. The oligonucleotide conjugate of any one of claims **35-42**, comprising at least one 2' modified nucleotide selected from locked nucleic acid (LNA) or ethylene nucleic acid (ENA).
44. The oligonucleotide conjugate of any one of the claims **35-43**, comprising at least one modified internucleotide linkage.
45. The oligonucleotide conjugate of any one of the claims **35-43**, comprising at least three modified internucleotide linkage.
46. The oligonucleotide conjugate of any one of the claims **35-44**, comprising at least one modified internucleotide linkage selected from a phosphorothioate, a phosphorodithioate, a methylphosphonate, a phosphotriester, or an amide linkage.
47. The oligonucleotide conjugate of claim **35**, wherein the compound of Formula (II-2) is located at the 3'-terminus of the oligonucleotide.
48. The oligonucleotide conjugate of claim **35**, wherein the compound of Formula (II-1) is located at an internal position within the oligonucleotide.
49. The oligonucleotide conjugate of any one of the claims **35-48**, wherein Formula (I) further comprises C to form A-B-C (Formula I-A), wherein A and C are not attached to B at the same terminus.
50. The oligonucleotide conjugate of claim **49**, wherein:
the compound of Formula (II-2) is located at the 5'-terminus of the oligonucleotide; and
the binding moiety is conjugated to the 3'-terminus of the oligonucleotide.
51. The oligonucleotide conjugate of claim **49**, wherein:
the compound of Formula (II-2) is located at the 3'-terminus of the oligonucleotide; and

- the binding moiety is conjugated to the 5'-terminus of the oligonucleotide.
52. The oligonucleotide conjugate of claim **49**, wherein:
the compound of Formula (II-1) is located in the interior of the of the oligonucleotide;
and
the binding moiety is conjugated to the 5'-terminus or the 3'-terminus of the oligonucleotide.
53. The oligonucleotide conjugate of any one of the claims **49-52**, wherein the binding moiety comprises an antibody or a binding fragment thereof.
54. The oligonucleotide conjugate of any one of the claims **49-53**, wherein the binding moiety comprises a humanized antibody or binding fragment thereof, a chimeric antibody or binding fragment thereof, a monoclonal antibody or binding fragment thereof, a monovalent Fab', a divalent Fab2, a single-chain variable fragment (scFv), a diabody, a minibody, a nanobody, a single-domain antibody (sdAb), or a camelid antibody or binding fragment thereof.
55. The oligonucleotide conjugate of claim **49**, wherein the binding moiety comprises a peptide or a small molecule.
56. The oligonucleotide conjugate of any one of the claims **35-55**, comprising from about 8 to about 50 nucleotides.
57. The oligonucleotide conjugate of any one of the claims **35-56**, comprising from about 10 to about 30 nucleotides.
58. The oligonucleotide conjugate of any one of the claims **35-57**, wherein B:
comprises an RNA oligonucleotide;
has a length from about 10 to about 30 nucleotides;
comprises at least one 2' modified nucleotide; and
comprises at least one modified internucleotide linkage.
59. The oligonucleotide conjugate of any one of the claims **35-58**, wherein B hybridizes to at least 8 contiguous bases of a target gene sequence.
60. The oligonucleotide conjugate of any one of the claims **35-59**, wherein B mediates RNA interference.
61. The oligonucleotide conjugate of any one of the claims **35-60**, wherein B is a sense strand.
62. The oligonucleotide conjugate of claim **61**, wherein B is hybridized with a second oligonucleotide to form a double-stranded oligonucleic acid molecule.
63. The oligonucleotide conjugate of claim **62**, wherein the second oligonucleotide is an antisense strand.

64. The oligonucleotide conjugate of claim **62** or **63**, wherein the second oligonucleotide is an RNA oligonucleotide.
65. The oligonucleotide conjugate of any one of the claims **58-64**, wherein the second oligonucleotide comprises at least one modification.
66. The oligonucleotide conjugate of any one of the claims **58-65**, wherein the second oligonucleotide comprises a compound of Formula (II) or a compound of Formula (IIa).
67. The oligonucleotide conjugate of claim **66**, wherein the second oligonucleotide comprises at least one 2' modified nucleotide.
68. The oligonucleotide conjugate of claim **66** or **67**, wherein the second oligonucleotide comprises at least one 2' modified nucleotide selected from 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide.
69. The oligonucleotide conjugate of any one of the claims **66-68**, wherein the second oligonucleotide comprises at least one 2' modified nucleotide selected from locked nucleic acid (LNA) or ethylene nucleic acid (ENA).
70. The oligonucleotide conjugate of any one of the claims **66-69**, wherein the second oligonucleotide comprises at least one modified internucleotide linkage.
71. The oligonucleotide conjugate of any one of the claims **66-70**, wherein the second oligonucleotide comprises at least one modified internucleotide linkage selected from a phosphorothioate linkage or a phosphorodithioate linkage.
72. The oligonucleotide conjugate of any claim 49, wherein C is a polymer.
73. The oligonucleotide conjugate of claim **72**, wherein the polymer comprises polyethylene glycol.
74. The oligonucleotide conjugate of any one of the claims **65-73**, wherein the oligonucleotide comprises a sense strand and an antisense strand:
 - wherein the sense strand is an RNA oligonucleotide;
 - is conjugated to a binding moiety, a polymer, or a combination thereof;
 - is from about 10 to about 30 nucleotides;
 - comprises at least one 2' modified nucleotide; and
 - comprises at least one modified internucleotide linkageand wherein the antisense strand:
 - is an RNA oligonucleotide;
 - is from about 10 to about 30 nucleotides;

comprises at least one 2' modified nucleotide; and

comprises at least one modified internucleotide linkage.

75. A method of treating a subject having a disease or condition characterized with a defective protein expression, comprising administering to the subject an oligonucleotide conjugate of claims **35-74** to modulate expression of a gene encoding the protein, thereby treating the disease or condition characterized with the defective protein expression.
76. A method of treating a subject having a disease or condition characterized with an overexpressed protein expression, comprising administering to the subject an oligonucleotide conjugate of claims **35-74** to modulate expression of a gene encoding the protein, thereby treating the disease or condition characterized with the overexpressed protein expression.
77. The method of claim **75** or **76**, wherein the disease or condition is a cancer.
78. The method of claim **75** or **76**, wherein the disease or condition is a neuromuscular disease, a muscle dystrophy, a muscle atrophy, a muscle wasting, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease.
79. The method of any one of the claims **75-78**, wherein the subject is a human.
80. A kit comprising a compound of claims **1-32** or an oligonucleotide conjugate of claims **35-74**.

FIG. 1A

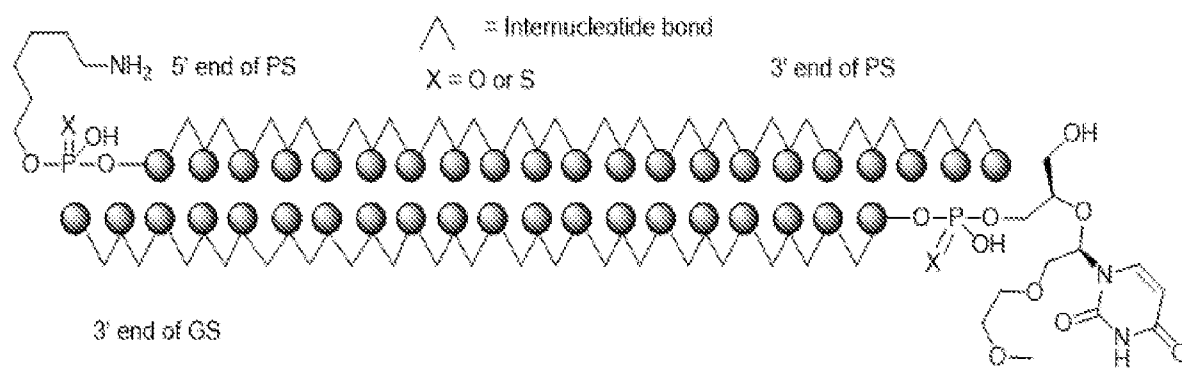


FIG. 1B

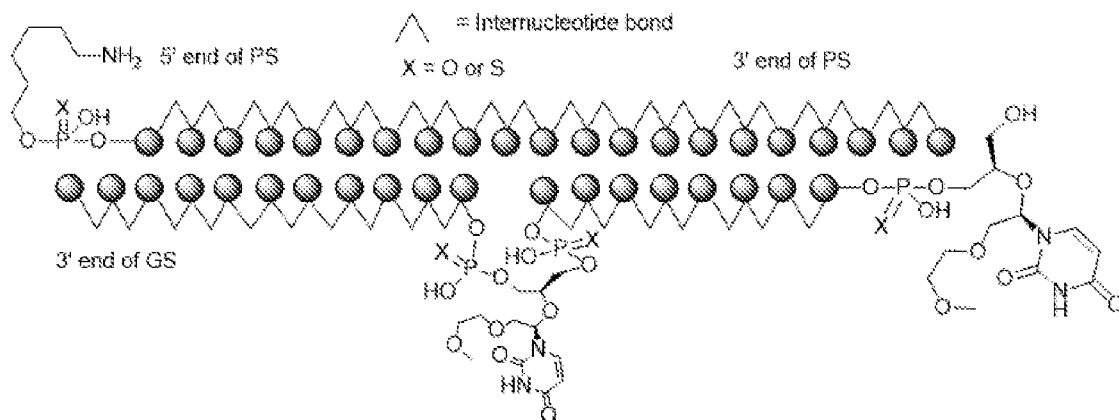


FIG. 2

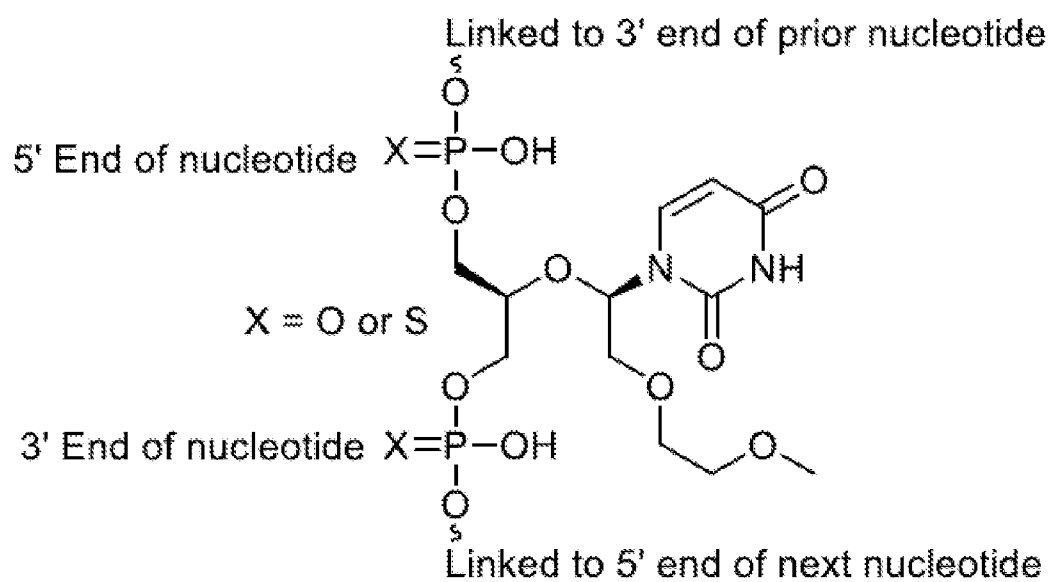


FIG. 3

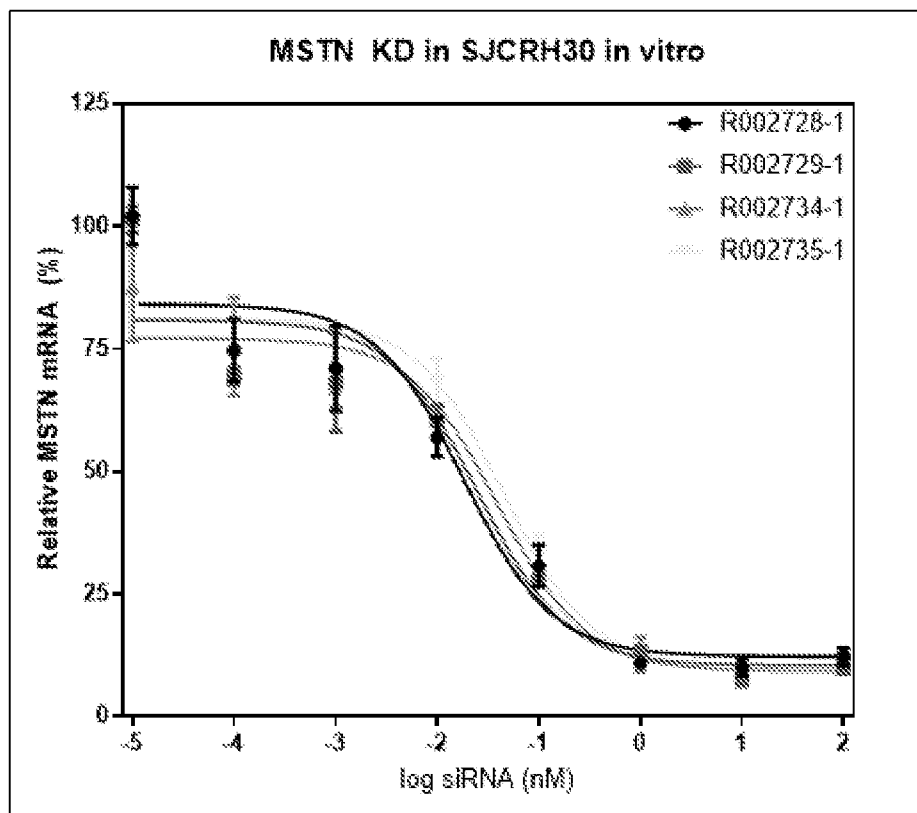


FIG. 4

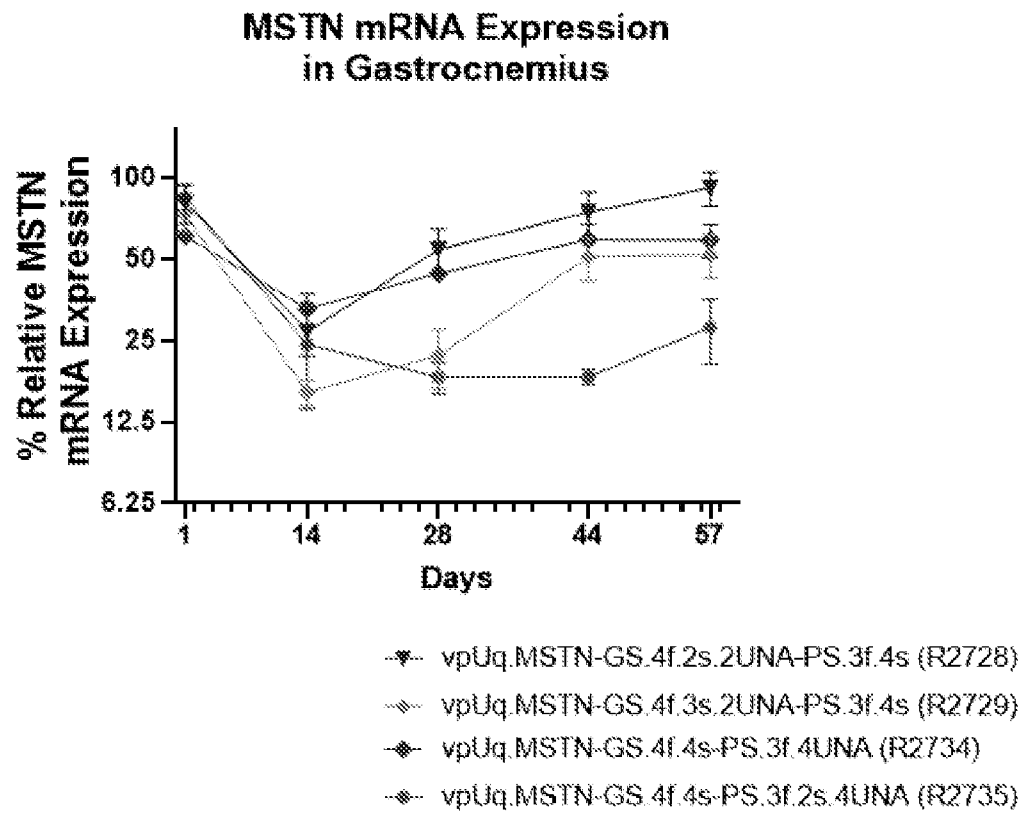


FIG. 1B

