NUCLEIC ACID-POLYPEPTIDE COMPOSITIONS AND USES THEREOF

CROSS-REFERENCE

[0001] This application claims benefit of U.S. Provisional Patent Application No. 62/568,238 filed on October 4, 2017, which is incorporated herein by reference in its entirety.

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.

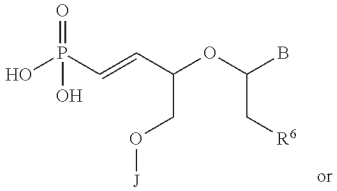
SUMMARY OF THE DISCLOSURE

[0003] Disclosed herein, in certain examples, are compositions and pharmaceutical formulations that comprise a binding moiety conjugated to a polynucleic acid molecule and a polymer. In some examples, 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 a polymer.

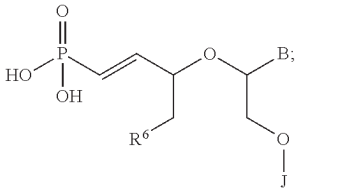
[0003a] In one aspect, provided herein is a polynucleotide conjugate molecule comprising a binding moiety and a polynucleotide,

wherein the polynucleotide comprises at least one 5′-vinylphosphonate modified non-natural nucleotide and at least one modified internucleotide linkage, or at least one inverted abasic moiety; and

wherein the at least one 5′-vinylphosphonate modified non-natural nucleotide is:



or



wherein B is a heterocyclic base moiety;

R^6 is selected from hydrogen, halogen, alkyl, alkoxy or aminoalkyl; and

J is an internucleotide linking group linking to an adjacent nucleotide of the polynucleotide.

[0004] Disclosed herein, in certain examples, is a molecule of Formula (I):

A-X-B-Y-C

Formula I

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or a first linker; and

Y is a bond or a second linker; and

wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide..

[0005] In some examples, the polynucleotide comprises a single strand. In some examples, the polynucleotide comprises two or more strands. In some examples, the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In some examples, the second polynucleotide comprises at least one modification.

[0006] In some examples, the first polynucleotide and the second polynucleotide are RNA molecules. In some examples, the first polynucleotide and the second polynucleotide are siRNA molecules.

[0007] In some examples, the first polynucleotide comprises a sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242. In some examples, the first polynucleotide consists of a sequence selected from SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242.

[0008] In some examples, the second polynucleotide comprises a sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242. In some examples, the second polynucleotide consists of a sequence selected from SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242.

[0009] In some examples, X and Y are independently a bond or a non-polymeric linker group. In some examples, X is a bond. In some examples, X is a C_1-C_6 alkyl group. In some examples, Y is a C_1-C_6 alkyl group. In some examples, X is a homobifuctional linker or a heterobifunctional linker, optionally conjugated to a C_1-C_6 alkyl group. In some examples, Y is a homobifuctional linker or a heterobifunctional linker.

[0010] In some examples, the binding moiety is an antibody or binding fragment thereof. In some examples, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, 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. In some examples, the antibody or binding fragment thereof is an anti-EGFR antibody or binding fragment thereof.

[0011] In some examples, C is polyethylene glycol. In some examples, C has a molecular weight of about 5000 Da.

[0012] In some examples, A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B. In some examples, Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B. In some examples, A-X, Y-C or a combination thereof is conjugated to an internucleotide linkage group.

[0013] In some examples, the molecule further comprises D. In some examples, D is conjugated to C or to A.

[0014] In some examples, D is conjugated to the molecule of Formula (I) according to Formula (II):

$$(A\text{-}X\text{-}B\text{-}Y\text{-}C_n)\text{-}L\text{-}D$$

Formula II

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or a first linker;

Y is a bond or a second linker;

L is a bond or a third linker;

D is an endosomolytic moiety; and

n is an integer between 0 and 1; and

wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide; and D is conjugated anywhere on A, B, or C.

[0015] In some examples, D is INF7 or melittin.

[0016] In some examples, D is an endosomolytic polymer.

[0017] In some examples, L is a C_1-C_6 alkyl group. In some examples, L is a homobifuctional linker or a heterobifunctional linker.

[0018] In some examples, the molecule further comprises at least a second binding moiety A. In some examples, the at least second binding moiety A is conjugated to A, to B, or to C. In some examples, the at least second binding moiety A is cholesterol.

[0019] In some examples, the molecule further comprises at least an additional polynucleotide B. In some examples, the at least an additional polynucleotide B is conjugated to A, to B, or to C.

[0020] In some examples, the molecule further comprises at least an additional polymer C. In some examples, the at least an additional polymer C is conjugated to A, to B, or to C.

[0021] Disclosed herein, in certain examples, is a molecule of Formula (I): A-X-B-Y-C (Formula I), wherein A is an antibody or its binding fragments thereof; B is a polynucleotide ; C is a polymer; X is a bond or first non-polymeric linker; and Y is a bond or second linker; wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide; and wherein A and C are not attached to B at the same terminus. In some examples, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some examples, the at least one inverted abasic moiety is at at least one terminus. In some examples, the polynucleotide comprises a single strand. In some examples, the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In some examples, the second polynucleotide comprises at least one modification. In some examples, the first polynucleotide and the second polynucleotide are RNA molecules. In some examples, the first polynucleotide comprises a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242. In some examples, the second polynucleotide comprises a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242. In some examples, Y is a non-polymeric linker group. In some examples, X is a bond. In some examples, X is a C_1-C_6 alkyl group. In some examples, Y is a C_1-C_6 alkyl group. In some examples, X is a homobifuctional linker or a heterobifunctional linker, optionally conjugated to a C_1-C_6 alkyl group. In some examples, Y is a homobifuctional linker or a heterobifunctional linker. In some examples, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, 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. In some examples, C is polyethylene glycol. In some examples, C has a molecular weight of about 1000 Da, 2000 Da, or 5000 Da. In some examples, A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B. In some examples, Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B. In some examples, the molecule further comprises D. In some examples, D is conjugated to C or to A. In some examples, D is conjugated to the molecule of Formula (I) according to Formula (II): $(A\text{-}X\text{-}B\text{-}Y\text{-}C_c)\text{-}L\text{-}D$ (Formula II), wherein A is an antibody or its binding fragments thereof; B is a polynucleotide; C is a polymer; X is a bond or first non-polymeric linker; Y is a bond or second linker; L is a bond or third linker; D is an endosomolytic moiety; and c is an integer between 0 and 1; wherein the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; wherein A and C are not attached to B at the same terminus; and wherein D is conjugated anywhere on A or C or to a terminus of B. In some examples, D is INF7 or melittin. In some examples, D is an endosomolytic polymer. In some examples, L is a C_1-C_6 alkyl group. In some examples, L is a homobifuctional linker or a heterobifunctional linker. In some examples, the molecule further comprises at least a second binding moiety. In some examples, the at least second binding moiety is conjugated to A, to B, or to C. In some examples, the at least second binding moiety is cholesterol. In some examples, the molecule further comprises at least an additional polynucleotide B. In some examples, the at least an additional polynucleotide B is conjugated to A, to B, or to C. In some examples, the molecule further comprises at least an additional polymer C. In some examples, the at least an additional polymer C is conjugated to A, to B, or to C.

[0022] Disclosed herein, in certain examples, is a pharmaceutical composition comprising a molecule described above, and a pharmaceutically acceptable excipient. In some examples, the pharmaceutical composition is formulated as a nanoparticle formulation. In some examples, the pharmaceutical composition is formulated for parenteral, oral, intranasal, buccal, rectal, or transdermal administration.

[0023] Disclosed herein, in certain examples, is a method of treating a disease or disorder in a patient in need thereof, comprising administering to the patient a composition comprising a molecule described above. In some examples, the disease or disorder is a cancer. In some examples, the cancer is a solid tumor. In some examples, the cancer is a hematologic malignancy. In some examples, the cancer comprises a KRAS-associated, an EGFR-associated, an AR-associated cancer, a β-catenin associated cancer, a PIK3C-associated cancer, or a MYC-associated cancer. In some examples, 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, or thyroid cancer. In some examples, the cancer comprises acute myeloid leukemia, CLL, DLBCL, or multiple myeloma. In some examples, the method is an immuno-oncology therapy.

[0024] Disclosed herein, in certain examples, 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 examples, the method is an *in vivo* method. In some examples, the patient is a human.

[0025] Disclosed herein, in certain examples, 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 examples, is a kit comprising a molecule described above.

BRIEF DECRIPTION OF THE DRAWINGS

[0027] **FIG. 1A** shows a cartoon representation of the structure of a 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs, as described in example molecular biology 10.

[0028] **FIG. 1B** shows a cartoon representation of the structure of a blunt ended duplex with 19 bases of complementarity and one 3' dinucleotide overhang, as described in molecular biology example 10.

[0029] **FIG. 2** shows a plot of Log (siRNA in nM) vs relative HPRT mRNA (%) for HCT Transfection with HPRT siRNA as described in molecular biology example 11.

[0030] **FIG. 3** shows a plot of Log (siRNA in nM) vs relative MSTN mRNA levels (% of untreated control) for SJCRH30 transfection with MSTN siRNAs as described in molecular biology example 12.

[0031] **FIG. 4** shows *in vivo* MSTN and HPRT mRNA downregulation in gastroc muscle, after IV administration of antibody siRNA conjugates, as described in molecular biology example 13.

[0032] **FIG. 5** shows *in vivo* MSTN mRNA downregulation in (A) gastroc, (B) quad and (C) heart muscle after IV administration of antibody siRNA conjugates, as described in molecular biology example 14.

[0033] **FIG. 6** shows *in vivo* HPRT mRNA downregulation in (A) gastroc and (B) liver after IV administration of antibody siRNA conjugates, as described in molecular biology example 15.

[0034] **FIG. 7** shows a plot of concentration (nM) vs %SSB mRNA as described in molecular biology example 16.

[0035] **FIG. 8** shows plots of siRNA concentration (nM) vs relative MSTN (figure 8A) and SSB (figure 8B) mRNA expression for SJCRH30 transfection, as described in molecular biology example 17.

[0036] **FIG. 9** shows plots of siRNA concentration (nM) vs relative MSTN (figure 8A) and SSB (figure 8B) mRNA expression, as described in molecular biology example 18.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0037] 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.

[0038] 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.

[0039] In some embodiments, described herein include a molecule those arrangement of the nucleic acid components effects intracellular uptake, stability, toxicity, efficacy, and/or non-specific immune stimulation. In some instances, the molecule comprises a binding moiety conjugated to a polynucleic acid molecule and a polymer. In some embodiments, the molecule comprises a molecule according to Formula (I): A-X-B-Y-C; in which A is a binding moiety, B is a polynucleotide comprising at least one 5'-vinylphosphonate modified nucleotide, C is a polymer, X is a bond or first linker, and Y is a bond or second linker. In some instances, the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide. In some instances, the molecule of Formula (I) further comprises D, an endosomolytic moiety.

[0040] In some embodiments, a molecule 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, a molecule 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 molecule comprises a molecule according to Formula (I): A-X-B-Y-C; in which A is a binding moiety, B is a polynucleotide comprising at least one 5'-vinylphosphonate modified nucleotide, C is a polymer, X is a bond or first linker, and Y is a bond or second linker. In some instances, the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide. In some instances, the molecule of Formula (I) further comprises D, an endosomolytic moiety.

[0041] In some embodiments, a molecule described herein is further used to treat a disease or disorder. In some instances, a molecule for the treatment of a disease or disorder is a molecule according to Formula (I): A-X-B-Y-C; in which A is a binding moiety, B is a polynucleotide comprising at least one 5'-vinylphosphonate modified nucleotide, C is a polymer, X is a bond or first linker, and Y is a bond or second linker. In some instances, the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide. In some instances, the molecule of Formula (I) further comprises D, an endosomolytic moiety.

[0042] In some embodiments, a molecule described herein is also used for inhibiting the expression of a target gene in a primary cell of a patient in need thereof. In such instances, a molecule for such use is a

molecule according to Formula (I): A-X-B-Y-C; in which A is a binding moiety, B is a polynucleotide comprising at least one 5'-vinylphosphonate modified nucleotide, C is a polymer, X is a bond or first linker, and Y is a bond or second linker. In some instances, the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety. In some instances, the molecule of Formula (I) further comprises D, an endosomolytic moiety.

[0043] In some embodiments, a molecule described herein is additionally used as an immuno-oncology therapy for the treatment of a disease or disorder. In some instance, the molecule is a molecule according to Formula (I): A-X-B-Y-C; in which A is a binding moiety, B is a polynucleotide comprising at least one 5'-vinylphosphonate modified nucleotide, C is a polymer, X is a bond or first linker, and Y is a bond or second linker. In some instances, the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety. In some instances, the molecule of Formula (I) further comprises D, an endosomolytic moiety.

[0044] In additional embodiments, described herein include a kit, which comprises one or more of the molecules described herein.

Therapeutic Molecule Platform

[0045] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a binding moiety conjugated to a polynucleic acid molecule comprising at least one 5'-vinylphosphonate modified nucleotide and a polymer. In some embodiments, a molecule (e.g., a therapeutic molecule) comprises a molecule according to Formula (I):

A-X-B-Y-C

Formula I

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or a first linker; and

Y is a bond or a second linker; and

wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide.

[0046] In some instances, the molecule of Formula (I) further comprises D, an endosomolytic moiety.

[0047] In some embodiments, at least one A and/or at least one C are conjugated to the 5' terminus of B, the 3' terminus of B, an internal site on B, or in any combinations thereof. In some instances, at least one A is conjugated at one terminus of B while at least one C is conjugated at the opposite terminus of B. In some instances, at least one of A is conjugated at one terminus of B while at least one of C is conjugated at an internal site on B.

[0048] In some cases, A and C are not conjugated or attached to B at the same terminus. In some cases, A is attached or conjugated to B at a first terminus of B. In some cases, C is attached or conjugated to B at a second terminus of B, and the second terminus of B is different than the first terminus. In some cases, A is attached or conjugated to B at the 5' terminus of B, and C is attached or conjugated to B at the 3' terminus of B. In other cases, A is attached or conjugated to B at the 3' terminus of B, and C is attached or conjugated to B at the 5' terminus of B.

[0049] In some embodiments, A is an antibody or binding fragment thereof. In some cases, C is a polymer. In some cases, A and C are not conjugated or attached to B at the same terminus. In some cases, A is attached or conjugated to B at a first terminus of B. In some cases, C is attached or conjugated to B at a second terminus of B, and the second terminus of B is different than the first terminus. In some cases, A is attached or conjugated to B at the 5' terminus of B, and C is attached or conjugated to B at the 3' terminus of B. In other cases, A is attached or conjugated to B at the 3' terminus of B, and C is attached or conjugated to B at the 5' terminus of B. In some cases, X which connects A to B is a bond or a non-polymeric linker. In some cases, X is a non-peptide linker (or a linker that does not comprise an amino acid residue). In some cases, Y which connects B to C is a bond or a second linker. In some instances, X connects A to the 5' terminus of B, and Y connects C to the 3' terminus of B. In other instances, X connects A to the 3' terminus of B, and Y connects C to the 5' terminus of B.

[0050] In some embodiments, X-B is conjugated or attached to the N-terminus, C-terminus, a constant region, a hinge region, or a Fc region of A. In some instances, X-B is conjugated or attached to the N-terminus of A. In some instances, X-B is conjugated or attached to the C-terminus of A. In some instances, X-B is conjugated or attached to a hinge region of A. In some instances, X-B is conjugated or attached to a constant region of A. In some instances, X-B is conjugated or attached to the Fc region of A.

[0051] In some instances, at least one B and/or at least one C, and optionally at least one D are conjugated to a first A. In some instances, the at least one B is conjugated at a terminus (e.g., a 5' terminus or a 3' terminus) to the first A or are conjugated via an internal site to the first A. In some cases, the at least one C is conjugated either directly to the first A or indirectly via the two or more Bs. If indirectly via the two or more Bs, the two or more Cs are conjugated either at the same terminus as the first A on B, at opposing terminus from the first A, or independently at an internal site. In some instances, at least one additional A is further conjugated to the first A, to B, or to C. In additional instances, the at least one D is optionally conjugated either directly or indirectly to the first A, to the at least one B, or to the at least one C. If directly to the first A, the at least one D is also optionally conjugated to the at least one B to form a A-D-B conjugate or is optionally conjugated to the at least one B and the at least one C to form a A-D-B-C conjugate. In some cases, the at least one additional A is different than the first A.

[0052] In some cases, two or more Bs and/or two or more Cs are conjugated to a first A. In some instances, the two or more Bs are conjugated at a terminus (e.g., a 5' terminus or a 3' terminus) to the first A or are conjugated via an internal site to the first A. In some instances, the two or more Cs are

conjugated either directly to the first A or indirectly via the two or more Bs. If indirectly via the two or more Bs, the two or more Cs are conjugated either at the same terminus as the first A on B, at opposing terminus from the first A, or independently at an internal site. In some instances, at least one additional A is further conjugated to the first A, to two or more Bs, or to two or more Cs. In additional instances, at least one D is optionally conjugated either directly or indirectly to the first A, to the two or more Bs, or to the two or more Cs. If indirectly to the first A, the at least one D is conjugated to the first A through the two or more Bs, through the two or more Cs, through a B-C orientation to form a A-B-C-D type conjugate, or through a C-B orientation to form a A-C-B-D type conjugate. In some cases, the at least one additional A is different than the first A. In some cases, the two or more Bs are different. In other cases, the two or more Bs are the same. In some instances, the two or more Cs are different. In other instances, the two or more Cs are the same. In additional instances, the two or more Ds are different. In additional instances, the two or more Ds are the same.

[0053] In other cases, two or more Bs and/or two or more Ds, optionally two or more Cs are conjugated to a first A. In some instances, the two or more Bs are conjugated at a terminus (e.g., a 5' terminus or a 3' terminus) to the first A or are conjugated via an internal site to the first A. In some instances, the two or more Ds are conjugated either directly to the first A or indirectly via the two or more Bs. If indirectly via the two or more Bs, the two or more Ds are conjugated either at the same terminus as the first A on B, at opposing terminus from the first A, or independently at an internal site. In some instances, at least one additional A is further conjugated to the first A, to the two or more Bs, or to the two or more Ds. In additional instances, the two or more Cs are optionally conjugated either directly or indirectly to the first A, to the two or more Bs, or to the two or more Ds. In some cases, the at least one additional A is different than the first A. In some cases, the two or more Bs are different. In other cases, the two or more Bs are the same. In some instances, the two or more Cs are different. In other instances, the two or more Cs are the same. In additional instances, the two or more Ds are different. In additional instances, the two or more Ds are the same.

[0054] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a molecule according to Formula (II):

$$(A\text{-}X\text{-}B\text{-}Y\text{-}C_c)\text{-}L\text{-}D$$

Formula II

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or a first linker;

Y is a bond or a second linker;

L is a bond or a third linker;

D is an endosomolytic moiety; and

c is an integer between 0 and 1; and

wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide; and D is conjugated anywhere on A, B, or C.

[0055] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a molecule according to Formula (III):

$$A_a\text{-}X\text{-}B_b\text{-}Y\text{-}C_c\text{-}L\text{-}D_n$$

Formula III

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

D is an endosomolytic moiety;

X is a bond or a first linker;

Y is a bond or a second linker;

L is a bond or a third linker;

a and b are independently an integer between 1-3;

c is an integer between 0 and 3; and

n is an integer between 0 and 10; and

wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide; A is conjugated anywhere on B, C, or D; B is conjugated anywhere on A, C, or D; C is conjugated anywhere on A, B, or D; and D is conjugated anywhere on A, B, or C.

[0056] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a molecule according to Formula (IIIa): A-X-B-L-D-Y-C.

[0057] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a molecule according to Formula (IIIb): $A_a\text{-}X\text{-}B_b\text{-}L\text{-}D_n$.

[0058] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a molecule according to Formula (IV):

$$A\text{-}X\text{-}(B_b\text{-}Y\text{-}C_c\text{-}L\text{-}D_n)_m$$

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

D is an endosomolytic moiety;

X is a bond or a first linker;

Y is a bond or a second linker;

L is a bond or a third linker;

a and b are independently an integer between 1-3;

c is an integer between 0 and 3;

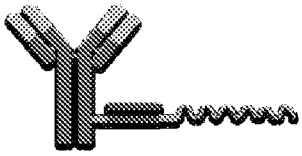
n is an integer between 0 and 10; and

m is an integer between 1-3; and

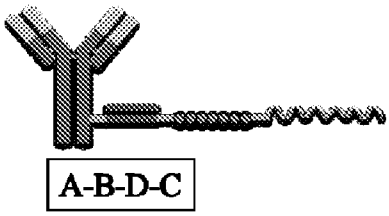
wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide; C is conjugated anywhere on B or D; and D is conjugated anywhere on B or C.

[0059] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a molecule according to Formula (IVa): $A\text{-}X\text{-}(B_b\text{-}L\text{-}D_n\text{-}Y\text{-}C_c)_m$.

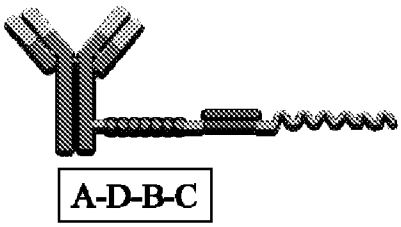
[0060] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



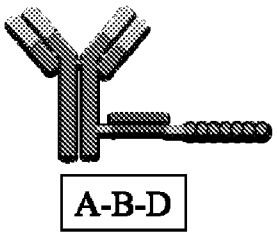
[0061] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



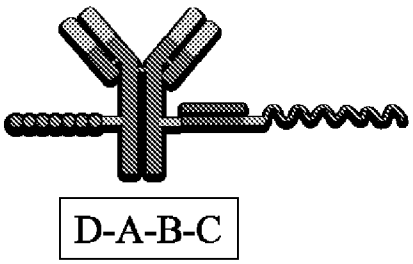
[0062] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



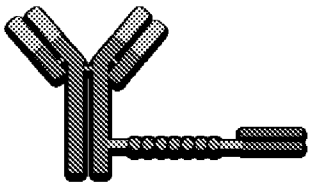
[0063] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



[0064] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:

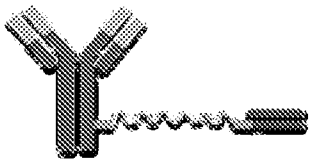


[0065] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



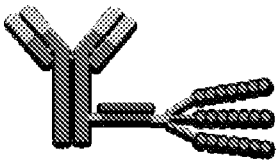
A-D-B

[0066] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



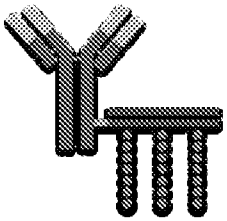
A-C-B

[0067] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



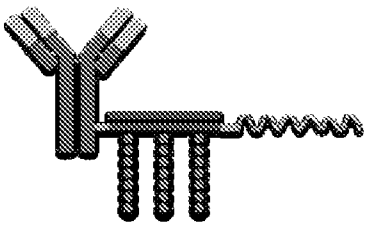
$A\text{-}B\text{-}(D)_n$

[0068] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



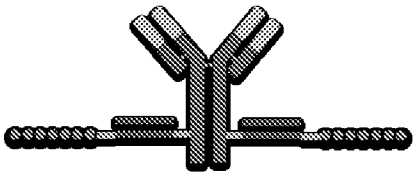
$A\text{-}B\text{-}(D)_n$

[0069] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:

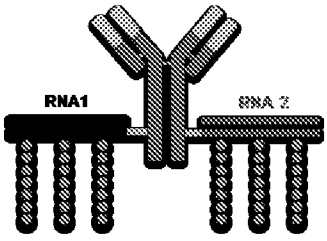


$A\text{-}B\text{-}(D)_n\text{-}C$

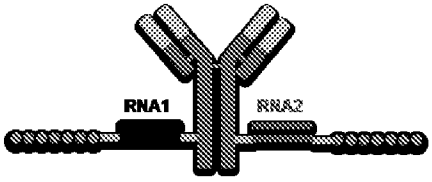
[0070] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



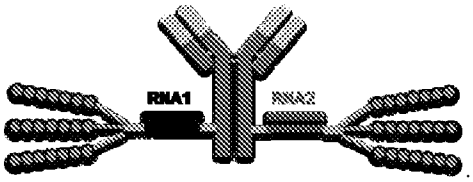
[0071] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



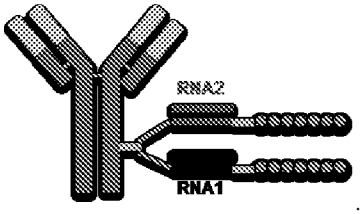
[0072] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



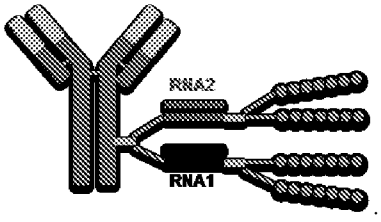
[0073] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



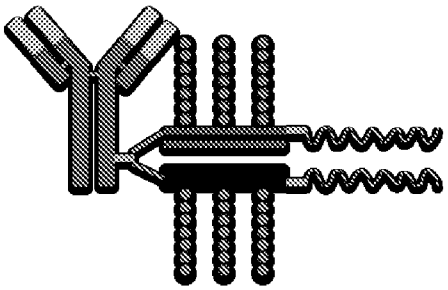
[0074] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



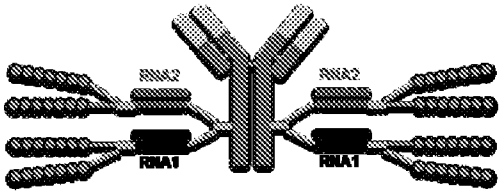
[0075] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



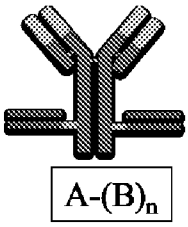
[0076] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:

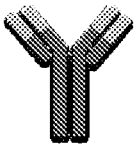


[0077] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



[0078] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



[0079] The  as illustrated above is for representation purposes only and encompasses a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, 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.

Polynucleic Acid Molecule Targets

[0080] 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.

[0081] 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/NRG, 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/NRG, RET, ROS, SKI, SRC, TIAM1,* or *TSC2*.

[0082] 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 *CNNTB1* (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*.

Polynucleic Acid Molecules That Target Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS)

[0083] Kirsten Rat Sarcoma Viral Oncogene Homolog (also known as GTPase KRas, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, or KRAS) is involved in regulating cell division. The K-Ras protein is a GTPase belonging to the Ras superfamily. In some instances, K-Ras modulates cell cycle progression, as well as induces growth arrest, apoptosis, and replicative senescence under different environmental triggers (*e.g.*, cellular stress, ultraviolet, heat shock, or ionizing irradiation). In some

cases, wild type *KRAS* gene has been shown to be frequently lost during tumor progression in different types of cancer, while mutations of *KRAS* gene have been linked to cancer development. In some instances, KRAS amplification has also been implicated in cancer development (*see, for example*, Valtorta *et al.* "KRAS gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy," *Int. J. Cancer* **133**: 1259-1266 (2013)). In such cases, the cancer pertains to a refractory cancer in which the patient has acquired resistance to a particular inhibitor or class of inhibitors.

[0084] In some embodiments, the *KRAS* gene is wild type or comprises a mutation. In some instances, *KRAS* mRNA is wild type or comprises a mutation. In some instances, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of wild type *KRAS* DNA or RNA. In some instances, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of *KRAS* DNA or RNA comprising a mutation (e.g., a substitution, a deletion, or an addition).

[0085] In some embodiments, *KRAS* DNA or RNA comprises one or more mutations. In some embodiments, *KRAS* DNA or RNA comprises one or more mutations at codons 12 or 13 in exon 1. In some instances, *KRAS* DNA or RNA comprises one or more mutations at codons 61, 63, 117, 119, or 146. In some instances, *KRAS* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 12, 13, 18, 19, 20, 22, 24, 26, 36, 59, 61, 63, 64, 68, 110, 116, 117, 119, 146, 147, 158, 164, 176, or a combination thereof of the KRAS polypeptide. In some embodiments, *KRAS* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues selected from G12V, G12D, G12C, G12A, G12S, G12F, G13C, G13D, G13V, A18D, L19F, T20R, Q22K, I24N, N26K, I36L, I36M, A59G, A59E, Q61K, Q61H, Q61L, Q61R, E63K, Y64D, Y64N, R68S, P110S, K117N, C118S, A146T, A146P, A146V, K147N, T158A, R164Q, K176Q, or a combination thereof of the KRAS polypeptide.

[0086] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *KRAS* DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *KRAS* DNA or RNA comprising one or more mutations at codons 12 or 13 in exon 1. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *KRAS* DNA or RNA comprising one or more mutations at codons 61, 63, 117, 119, or 146. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *KRAS* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 12, 13, 18, 19, 20, 22, 24, 26, 36, 59, 61, 63, 64, 68, 110, 116, 117, 119, 146, 147, 158, 164, 176, or a combination thereof of the KRAS polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *KRAS* DNA or RNA comprising one or more mutations corresponding to amino acid residues selected from G12V, G12D, G12C, G12A, G12S, G12F, G13C, G13D, G13V, A18D, L19F, T20R, Q22K, I24N, N26K, I36L, I36M, A59G, A59E, Q61K, Q61H, Q61L, Q61R, E63K, Y64D, Y64N, R68S, P110S, K117N, C118S, A146T, A146P, A146V, K147N, T158A, R164Q, K176Q, or a combination thereof of the KRAS polypeptide.

Polynucleic Acid Molecules That Target Epidermal Growth Factor Receptor (EGFR)

[0087] Epidermal growth factor receptor (EGFR, ErbB-1, or HER1) is a transmembrane tyrosine kinase receptor and a member of the ErbB family of receptors, which also include HER2/c-neu (ErbB-2), Her3 (ErbB-3) and Her4 (ErbB-4). In some instances, EGFR mutations drive the downstream activation of RAS/RAF/MAPK, PI3K/AKT, and/or JAK/STAT pathways, leading to mitosis, cell proliferation, and suppression of apoptosis. In addition, amplification of wild-type EGFR gene has been implicated in the development of cancers such as glioblastomas and non-small cell lung cancer (Talasila, et al., "EGFR Wild-type Amplification and Activation Promote Invasion and Development of Glioblastoma Independent of Angiogenesis," Acta Neuropathol. 125(5): 683-698 (2013); Bell *et al.*, "Epidermal Growth Factor Receptor Mutations and Gene Amplification in Non–Small-Cell Lung Cancer: Molecular Analysis of the IDEAL/INTACT Gefitinib Trials," *J. Clinical Oncology* **23**(31): 8081-8092 (2005)).

[0088] In some embodiments, *EGFR* DNA or RNA is wild type *EGFR* or *EGFR* comprising a mutation. In some instances, *EGFR* is wild type *EGFR*. In some instances, *EGFR* DNA or RNA comprises a mutation. In some instances, the polynucleic acid molecule hybridizes to a target region of wild type *EGFR* DNA or RNA. In some instances, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising a mutation (e.g., a substitution, a deletion, or an addition).

[0089] In some instances, *EGFR* DNA or RNA comprises one or more mutations. In some embodiments, *EGFR* DNA or RNA comprises one or more mutations within one or more exons. In some instances, the one or more exons comprise exon 18, exon 19, exon 20, exon 21 or exon 22. In some instances, EGFR DNA or RNA comprises one or more mutations in exon 18, exon 19, exon 20, exon 21, exon 22 or a combination thereof.

[0090] In some instances, *EGFR* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 34, 38, 45, 62, 63, 77, 78, 108, 114, 120, 140, 148, 149, 160, 177, 178, 189, 191, 198, 220, 222, 223, 229, 237, 240, 244, 252, 254, 255, 256, 263, 270, 273, 276, 282, 288, 289, 301, 303, 304, 309, 314, 326, 331, 354, 363, 373, 337, 380, 384, 393, 427, 428, 437, 441, 447, 465, 475, 515, 526, 527, 531, 536, 541, 546, 571, 588, 589, 596, 596, 598, 602, 614, 620, 628, 636, 641, 645, 651, 671, 689, 694, 700, 709, 712, 714, 715, 716, 719, 720, 721, 731, 733, 739-744, 742, 746-750, 746-752, 746, 747, 747-749, 747-751, 747-753, 751, 752, 754, 752-759, 750, 761-762, 761, 763, 765, 767-768, 767-769, 768, 769, 769-770, 770-771, 772, 773-774, 773, 774, 774-775, 776, 779, 783, 784, 786, 790, 792, 794, 798, 803, 805, 807, 810, 826, 827, 831, 832, 833, 835, 837, 838, 839, 842, 843, 847, 850, 851, 853, 854, 856, 858, 861, 863, 894, 917, 967, 1006, 1019, 1042, 1100, 1129, 1141, 1153, 1164, 1167, or a combination thereof of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 747, 761, 790, 854, 858, or a combination thereof of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 761, 790, 858, or a combination thereof of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises a mutation at a position corresponding to amino acid residue 747 of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises a mutation at a position corresponding to amino acid residue 761 of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises a mutation

at a position corresponding to amino acid residue 790 of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises a mutation at a position corresponding to amino acid residue 854 of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises a mutation at a position corresponding to amino acid residue 858 of the EGFR polypeptide.

[0091] In some embodiments, *EGFR* DNA or RNA comprises one or more mutations selected from T34M, L38V, E45Q, L62R, G63R, G63K, S77F, F78L, R108K, R108G, E114K, A120P, L140V, V148M, R149W, E160K, S177P, M178I, K189T, D191N, S198R, S220P, R222L, R222C, S223Y, S229C, A237Y, C240Y, R244G, R252C, R252P, F254I, R255 (nonsense mutation), D256Y, T263P, Y270C, T273A, Q276 (nonsense), E282K, G288 (frame shift), A289D, A289V, A289T, A289N, A289D, V301 (deletion), D303H, H304Y, R309Q, D314N, C326R, G331R, T354M, T363I, P373Q, R337S, S380 (frame shift), T384S, D393Y, R427L, G428S, S437Y, V441I, S447Y, G465R, I475V, C515S, C526S, R527L, R531 (nonsense), V536M, L541I, P546Q, C571S, G588S, P589L, P596L, P596S, P596R, P596L, G598V, G598A, E602G, G614D, C620Y, C620W, C628Y, C628F, C636Y, T638M, P641H, S645C, V651M, R671C, V689M, P694S, N700D, E709A, E709K, E709Q, E709K, F712L, K714N, I715S, K716R, G719A, G719C, G719D, G719S, S720C, S720F, G721V, W731Stop, P733L, K739-I744 (insertion), V742I, V742A, E746-A750 (deletion), E746K, L747S, L747-E749 (deletion), L747-T751 (deletion), L747-P753 (deletion), G746-S752 (deletion), T751I, S752Y, K754 (deletion), S752-I759 (deletion), A750P, D761-E762 (e.g., residues EAFQ insertion (SEQ ID NO: 2110)), D761N, D761Y, A763V, V765A, A767-S768 (e.g., residues TLA insertion), A767-V769 (e.g., residues ASV insertion), S768I, S768T, V769L, V769M, V769-D770 (e.g., residue Y insertion), 770-771 (e.g., residues GL insertion), 770-771 (e.g., residue G insertion), 770-771 (e.g., residues CV insertion), 770-771 (e.g., residues SVD insertion), P772R, 773-774 (e.g., residues NPH insertion), H773R, H773L, V774M, 774-775 (e.g., residues HV insertion), R776H, R776C, G779F, T783A, T784F, T854A, V786L, T790M, L792P, P794H, L798F, R803W, H805R, D807H, G810S, N826S, Y827 (nonsense), R831H, R832C, R832H, L833F, L833V, H835L, D837V, L838M, L838P, A839V, N842H, V843L, T847K, T847I, H850N, V851A, I853T, F856L, L858R, L858M, L861Q, L861R, G863D, Q894L, G917A, E967A, D1006Y, P1019L, S1042N, R1100S, H1129Y, T1141S, S1153I, Q1164R, L1167M, or a combination thereof of the EGFR polypeptide.

[0092] In some instances, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations in exon 18, exon 19, exon 20, exon 21, exon 22 or a combination thereof.

[0093] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 34, 38, 45, 62, 63, 77, 78, 108, 114, 120, 140, 148, 149, 160, 177, 178, 189, 191, 198, 220, 222, 223, 229, 237, 240, 244, 252, 254, 255, 256, 263, 270, 273, 276, 282, 288, 289, 301, 303, 304, 309, 314, 326, 331, 354, 363, 373, 337, 380, 384, 393, 427, 428, 437, 441, 447, 465, 475, 515, 526, 527, 531, 536, 541, 546, 571, 588, 589, 596, 596, 598, 602, 614, 620, 628, 636, 641, 645, 651, 671, 689, 694, 700, 709, 712, 714,

715, 716, 719, 720, 721, 731, 733, 739-744, 742, 746-750, 746-752, 746, 747, 747-749, 747-751, 747-753, 751, 752, 754, 752-759, 750, 761-762, 761, 763, 765, 767-768, 767-769, 768, 769, 769-770, 770-771, 772, 773-774, 773, 774, 774-775, 776, 779, 783, 784, 786, 790, 792, 794, 798, 803, 805, 807, 810, 826, 827, 831, 832, 833, 835, 837, 838, 839, 842, 843, 847, 850, 851, 853, 854, 856, 858, 861, 863, 894, 917, 967, 1006, 1019, 1042, 1100, 1129, 1141, 1153, 1164, 1167, or a combination thereof of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 747, 761, 790, 854, 858, or a combination thereof of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 761, 790, 858, or a combination thereof of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising a mutation at a position corresponding to amino acid residue 747 of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising a mutation at a position corresponding to amino acid residue 761 of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising a mutation at a position corresponding to amino acid residue 790 of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising a mutation at a position corresponding to amino acid residue 854 of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising a mutation at a position corresponding to amino acid residue 858 of the EGFR polypeptide.

[0094] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations selected from T34M, L38V, E45Q, L62R, G63R, G63K, S77F, F78L, R108K, R108G, E114K, A120P, L140V, V148M, R149W, E160K, S177P, M178I, K189T, D191N, S198R, S220P, R222L, R222C, S223Y, S229C, A237Y, C240Y, R244G, R252C, R252P, F254I, R255 (nonsense mutation), D256Y, T263P, Y270C, T273A, Q276 (nonsense), E282K, G288 (frame shift), A289D, A289V, A289T, A289N, A289D, V301 (deletion), D303H, H304Y, R309Q, D314N, C326R, G331R, T354M, T363I, P373Q, R337S, S380 (frame shift), T384S, D393Y, R427L, G428S, S437Y, V441I, S447Y, G465R, I475V, C515S, C526S, R527L, R531 (nonsense), V536M, L541I, P546Q, C571S, G588S, P589L, P596L, P596S, P596R, P596L, G598V, G598A, E602G, G614D, C620Y, C620W, C628Y, C628F, C636Y, T638M, P641H, S645C, V651M, R671C, V689M, P694S, N700D, E709A, E709K, E709Q, E709K, F712L, K714N, I715S, K716R, G719A, G719C, G719D, G719S, S720C, S720F, G721V, W731Stop, P733L, K739-I744 (insertion), V742I, V742A, E746-A750 (deletion), E746K, L747S, L747-E749 (deletion), L747-T751 (deletion), L747-P753 (deletion), G746-S752 (deletion), T751I, S752Y, K754 (deletion), S752-I759 (deletion), A750P, D761-E762 (e.g., residues EAFQ insertion (SEQ ID NO: 2110)), D761N, D761Y, A763V, V765A, A767-S768 (e.g., residues TLA insertion), A767-V769 (e.g., residues ASV insertion), S768I, S768T, V769L, V769M, V769-D770 (e.g., residue Y insertion), 770-771 (e.g., residues GL insertion), 770-771 (e.g., residue G

insertion), 770-771 (e.g., residues CV insertion), 770-771 (e.g., residues SVD insertion), P772R, 773-774 (e.g., residues NPH insertion), H773R, H773L, V774M, 774-775 (e.g., residues HV insertion), R776H, R776C, G779F, T783A, T784F, T854A, V786L, T790M, L792P, P794H, L798F, R803W, H805R, D807H, G810S, N826S, Y827 (nonsense), R831H, R832C, R832H, L833F, L833V, H835L, D837V, L838M, L838P, A839V, N842H, V843L, T847K, T847I, H850N, V851A, I853T, F856L, L858R, L858M, L861Q, L861R, G863D, Q894L, G917A, E967A, D1006Y, P1019L, S1042N, R1100S, H1129Y, T1141S, S1153I, Q1164R, L1167M, or a combination thereof of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations selected from L747S, D761Y, T790M, T854A, L858R, or a combination thereof of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations selected from D761Y, T790M, L858R, or a combination thereof of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising mutation L747S of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising mutation D761Y of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising mutation T790M of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising mutation T854A of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising mutation L858R of the EGFR polypeptide.

Polynucleic Acid Molecules That Target Androgen Receptor (AR)

[0095] Androgen receptor (AR) (also known as NR3C4, nuclear receptor subfamily 3, group C, gene 4) belongs to the steroid hormone group of nuclear receptor superfamily along with related members: estrogen receptor (ER), glucocorticoid receptor (GR), progesterone receptor (PR), and mineralocorticoid receptor (MR). Androgens, or steroid hormones, modulate protein synthesis and tissue remodeling through the androgen receptor. The AR protein is a ligand-inducible zinc finger transcription factor that regulates target gene expression. The presence of mutations in the *AR* gene has been observed in several types of cancers (e.g., prostate cancer, breast cancer, bladder cancer, or esophageal cancer), and in some instances, has been linked to metastatic progression.

[0096] In some embodiments, *AR* DNA or RNA is wild type or comprises one or more mutations and/or splice variants. In some instances, *AR* DNA or RNA comprises one or more mutations. In some instances, *AR* DNA or RNA comprises one or more splice variants selected from AR splice variants including but not limited to AR1/2/2b, ARV2, ARV3, ARV4, AR1/2/3/2b, ARV5, ARV6, ARV7, ARV9, ARV10, ARV11, ARV12, ARV13, ARV14, ARV15, ARV16, and ARV(v567es). In some instances, the polynucleic acid molecule hybridizes to a target region of *AR* DNA or RNA comprising a mutation (e.g., a substitution, a deletion, or an addition) or a splice variant.

[0097] In some embodiments, *AR* DNA or RNA comprises one or more mutations. In some embodiments, *AR* DNA or RNA comprises one or more mutations within one or more exons. In some instances, the one or more exons comprise exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, or exon 8. In some embodiments, *AR* DNA or RNA comprises one or more mutations within exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8 or a combination thereof. In some instances, AR DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 2, 14, 16, 29, 45, 54, 57, 64, 106, 112, 176, 180, 184, 194, 198, 204, 214, 221, 222, 233, 243, 252, 255, 266, 269, 287, 288, 334, 335, 340, 363, 368, 369, 390, 403, 443, 491, 505, 513, 524, 524, 528, 533, 547, 548, 564, 567, 568, 574, 547, 559, 568, 571, 573, 575, 576, 577, 578, 579, 580, 581, 582, 585, 586, 587, 596, 597, 599, 601, 604, 607, 608, 609, 610, 611, 615, 616, 617, 619, 622, 629, 630, 638, 645, 647, 653, 662, 664, 670, 671, 672, 674, 677, 681, 682, 683, 684, 687, 688, 689, 690, 695, 700, 701, 702, 703, 705, 706, 707, 708, 710, 711, 712, 715, 717, 720, 721, 722, 723, 724, 725, 726, 727, 728, 730, 732, 733, 737, 739, 741, 742, 743, 744, 745, 746, 748, 749, 750, 751, 752, 754, 755, 756, 757, 758, 759, 762, 763, 764, 765, 766, 767, 768, 771, 772, 774, 777, 779, 786, 795, 780, 782, 784, 787, 788, 790, 791, 793, 794, 798, 802, 803, 804, 806, 807, 812, 813, 814, 819, 820, 821, 824, 827, 828, 830, 831, 834, 840, 841, 842, 846, 854, 855, 856, 863, 864, 866, 869, 870, 871, 874, 875, 877, 879, 880, 881, 886, 888, 889, 891, 892, 895, 896, 897, 898, 902, 903, 904, 907, 909, 910, 911, 913, 916, 919, or a combination thereof of the AR polypeptide. In some embodiments, AR DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues selected from E2K, P14Q, K16N, V29M, S45T, L54S, L57Q, Q64R, Y106C, Q112H, S176S, K180R, L184P, Q194R, E198G, G204S, G214R, K221N, N222D, D233K, S243L, A252V, L255P, M266T, P269S, A287D, E288K, S334P, S335T, P340L, Y363N, L368V, A369P, P390R, P390S, P390L, A403V, Q443R, G491S, G505D, P513S, G524D, G524S, D528G, P533S, L547F, P548S, D564Y, S567F, G568W, L574P, L547F, C559Y, G568W, G568V, Y571C, Y571H, A573D, T575A, C576R, C576F, G577R, S578T, C579Y, C579F, K580R, V581F, F582Y, F582S, R585K, A586V, A587S, A596T, A596S, S597G, S597I, N599Y, C601F, D604Y, R607Q, R608K, K609N, D610T, C611Y, R615H, R615P, R615G, R616C, L616R, L616P, R617P, C619Y, A622V, R629W, R629Q, K630T, L638M, A645D, S647N, E653K, S662 (nonsense), I664N, Q670L, Q670R, P671H, I672T, L674P, L677P, E681L, P682T, G683A, V684I, V684A, A687V, G688Q, H689P, D690V, D695N, D695V, D695H, L700M, L701P, L701I, H701H, S702A, S703G, N705S, N705Y, E706 (nonsense), L707R, G708A, R710T, Q711E, L712F, V715M, K717Q, K720E, A721T, L722F, P723S, G724S, G724D, G724N, F725L, R726L, N727K, L728S, L728I, V730M, D732N, D732Y, D732E, Q733H, I737T, Y739D, W741R, M742V, M742I, G743R, G743V, L744F, M745T, V746M, A748D, A748V, A748T, M749V, M749I, G750S, G750D, W751R, R752Q, F754V, F754L, T755A, N756S, N756D, V757A, N758T, S759F, S759P, L762F, Y763H, Y763C, F764L, A765T, A765V, P766A, P766S, D767E, L768P, L768M, N771H, E772G, E772A, R774H, R774C, K777T, R779W, R786Q, G795V, M780I, S782N, C784Y, M787V, R788S, L790F, S791P, E793D, F794S, Q798E, Q802R, G803L, F804L, C806Y, M807V, M807R, M807I, L812P, F813V, S814N, N819Q, G820A, L821V, Q824L, Q824R, F827L, F827V, D828H, L830V, L830P, R831Q, R831L, Y834C, R840C, R840H,

I841S, I842T, R846G, R854K, R855C, R855H, F856L, L863R, D864N, D864E, D864G, V866L, V866M, V866E, I869M, A870G, A870V, R871G, H874Y, H874R, Q875K, T877S, T877A, D879T, D879G, L880Q, L881V, M886V, S888L, V889M, F891L, P892L, M895T, A896T, E897D, I898T, Q902R, V903M, P904S, P904H, L907F, G909R, G909E, K910R, V911L, P913S, F916L, Q919R, or a combination thereof of the AR polypeptide.

[0098] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *AR* DNA or RNA comprising one or more mutations. In some embodiments the polynucleic acid hybridizes to one or more AR splice variants. In some embodiments the polynucleic acid hybridizes to *AR* DNA or RNA comprising one or more AR splice variants including but not limited to AR1/2/2b, ARV2, ARV3, ARV4, AR1/2/3/2b, ARV5, ARV6, ARV7, ARV9, ARV10, ARV11, ARV12, ARV13, ARV14, ARV15, ARV16, and ARV(v567es). In some embodiments, the polynucleic acid molecule hybridizes to a target region of *AR* DNA or RNA comprising one or more mutations within exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8 or a combination thereof. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *AR* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 2, 14, 16, 29, 45, 54, 57, 64, 106, 112, 176, 180, 184, 194, 198, 204, 214, 221, 222, 233, 243, 252, 255, 266, 269, 287, 288, 334, 335, 340, 363, 368, 369, 390, 403, 443, 491, 505, 513, 524, 524, 528, 533, 547, 548, 564, 567, 568, 574, 547, 559, 568, 571, 573, 575, 576, 577, 578, 579, 580, 581, 582, 585, 586, 587, 596, 597, 599, 601, 604, 607, 608, 609, 610, 611, 615, 616, 617, 619, 622, 629, 630, 638, 645, 647, 653, 662, 664, 670, 671, 672, 674, 677, 681, 682, 683, 684, 687, 688, 689, 690, 695, 700, 701, 702, 703, 705, 706, 707, 708, 710, 711, 712, 715, 717, 720, 721, 722, 723, 724, 725, 726, 727, 728, 730, 732, 733, 737, 739, 741, 742, 743, 744, 745, 746, 748, 749, 750, 751, 752, 754, 755, 756, 757, 758, 759, 762, 763, 764, 765, 766, 767, 768, 771, 772, 774, 777, 779, 786, 795, 780, 782, 784, 787, 788, 790, 791, 793, 794, 798, 802, 803, 804, 806, 807, 812, 813, 814, 819, 820, 821, 824, 827, 828, 830, 831, 834, 840, 841, 842, 846, 854, 855, 856, 863, 864, 866, 869, 870, 871, 874, 875, 877, 879, 880, 881, 886, 888, 889, 891, 892, 895, 896, 897, 898, 902, 903, 904, 907, 909, 910, 911, 913, 916, 919, or a combination thereof of the AR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *AR* DNA or RNA comprising one or more mutations selected from E2K, P14Q, K16N, V29M, S45T, L54S, L57Q, Q64R, Y106C, Q112H, S176S, K180R, L184P, Q194R, E198G, G204S, G214R, K221N, N222D, D233K, S243L, A252V, L255P, M266T, P269S, A287D, E288K, S334P, S335T, P340L, Y363N, L368V, A369P, P390R, P390S, P390L, A403V, Q443R, G491S, G505D, P513S, G524D, G524S, D528G, P533S, L547F, P548S, D564Y, S567F, G568W, L574P, L547F, C559Y, G568W, G568V, Y571C, Y571H, A573D, T575A, C576R, C576F, G577R, S578T, C579Y, C579F, K580R, V581F, F582Y, F582S, R585K, A586V, A587S, A596T, A596S, S597G, S597I, N599Y, C601F, D604Y, R607Q, R608K, K609N, D610T, C611Y, R615H, R615P, R615G, R616C, L616R, L616P, R617P, C619Y, A622V, R629W, R629Q, K630T, L638M, A645D, S647N, E653K, S662 (nonsense), I664N, Q670L, Q670R, P671H, I672T, L674P, L677P, E681L, P682T, G683A, V684I, V684A, A687V, G688Q, H689P, D690V, D695N, D695V, D695H, L700M, L701P, L701I, H701H, S702A, S703G, N705S, N705Y, E706 (nonsense), L707R, G708A,

R710T, Q711E, L712F, V715M, K717Q, K720E, A721T, L722F, P723S, G724S, G724D, G724N, F725L, R726L, N727K, L728S, L728I, V730M, D732N, D732Y, D732E, Q733H, I737T, Y739D, W741R, M742V, M742I, G743R, G743V, L744F, M745T, V746M, A748D, A748V, A748T, M749V, M749I, G750S, G750D, W751R, R752Q, F754V, F754L, T755A, N756S, N756D, V757A, N758T, S759F, S759P, L762F, Y763H, Y763C, F764L, A765T, A765V, P766A, P766S, D767E, L768P, L768M, N771H, E772G, E772A, R774H, R774C, K777T, R779W, R786Q, G795V, M780I, S782N, C784Y, M787V, R788S, L790F, S791P, E793D, F794S, Q798E, Q802R, G803L, F804L, C806Y, M807V, M807R, M807I, L812P, F813V, S814N, N819Q, G820A, L821V, Q824L, Q824R, F827L, F827V, D828H, L830V, L830P, R831Q, R831L, Y834C, R840C, R840H, I841S, I842T, R846G, R854K, R855C, R855H, F856L, L863R, D864N, D864E, D864G, V866L, V866M, V866E, I869M, A870G, A870V, R871G, H874Y, H874R, Q875K, T877S, T877A, D879T, D879G, L880Q, L881V, M886V, S888L, V889M, F891L, P892L, M895T, A896T, E897D, I898T, Q902R, V903M, P904S, P904H, L907F, G909R, G909E, K910R, V911L, P913S, F916L, Q919R, or a combination thereof of the AR polypeptide.

Polynucleic Acid Molecules That Target B-catenin and B-catenin-associated Genes

[0099] Catenin beta-1 (also known as CTNNB1, β-catenin, or beta-catenin) is a member of the catenin protein family. In humans, it is encoded by the *CTNNB1* gene and is known for its dual functions – cell-cell adhesion and gene transcription. Beta-catenin is an integral structural component of cadherin-based adherens junctions and regulates cell growth and adhesion between cells and anchors the actin cytoskeleton. In some instance, beta-catenin is responsible for transmitting the contact inhibition signal that causes the cells to stop dividing once the epithelial sheet is complete. Beta-catenin is also a key nuclear effector of the Wnt signaling pathway. In some instances, imbalance in the structural and signaling properties of beta-catenin results in diseases and deregulated growth connected to malignancies such as cancer. For example, overexpression of beta-catenin has been linked to cancers such as gastric cancer (Suriano, et al., "Beta-catenin (CTNNB1) gene amplification: a new mechanism of protein overexpression in cancer," Genes Chromosomes Cancer 42(3): 238-246 (2005)). In some cases, mutations in *CTNNB1* gene have been linked to cancer development (e.g., colon cancer, melanoma, hepatocellular carcinoma, ovarian cancer, endometrial cancer, medulloblastoma pilomatricomas, or prostrate cancer), and in some instances, has been linked to metastatic progression. In additional cases, mutations in the *CTNNB1* gene cause beta-catenin to translocate to the nucleus without any external stimulus and drive the transcription of its target genes continuously. In some cases, the potential of beta-catenin to change the previously epithelial phenotype of affected cells into an invasive, mesenchyme-like type contributes to metastasis formation.

[0100] In some embodiments, *CTNNB1* gene is wild type *CTNNB1* or *CTNNB1* comprising one or more mutations. In some instances, *CTNNB1* is wild type *CTNNB1*. In some instances, *CTNNB1* is *CTNNB1* comprising one or more mutations. In some instances, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of wild type *CTNNB1*. In some instances, the

polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of *CTNNB1* comprising a mutation (e.g., a substitution, a deletion, or an addition).

[0101] In some embodiments, *CTNNB1* DNA or RNA comprises one or more mutations. In some embodiments, *CTNNB1* DNA or RNA comprises one or more mutations within one or more exons. In some instances, the one or more exons comprise exon 3. In some instances, *CTNNB1* DNA or RNA comprises one or more mutations at codons 32, 33, 34, 37, 41, 45, 183, 245, 287 or a combination thereof. In some instances, *CTNNB1* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 25, 31, 32, 33, 34, 35, 36, 37, 41, 45, 140, 162, 170, 199, 213, 215, 257, 303, 322, 334, 354, 367, 373, 383, 387, 402, 426, 453, 474, 486, 515, 517, 535, 553, 555, 582, 587, 619, 623, 641, 646, 688, 703, 710, 712, 714, 724, 738, 777, or a combination thereof of the CTNNB1 polypeptide. In some embodiments, *CTNNB1* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues selected from W25 (nonsense mutation), L31M, D32A, D32N, D32Y, D32G, D32H, S33C, S33Y, S33F, S33P, G34R, G34E, G34V, I35S, H36Y, S37F, S37P, S37C, S37A, T41N, T41A, T41I, S45Y, S45F, S45C, I140T, D162E, K170M, V199I, C213F, A215T, T257I, I303M, Q322K, E334K, K354T, G367V, P373S, W383G, N387K, L402F, N426D, R453L, R453Q, R474 (nonsense mutation), R486C, R515Q, L517F, R535 (nonsense mutation), R535Q, M553V, G555A, R582Q, R587Q, C619Y, Q623E, T641 (frame shift), S646F, M688T, Q703H, R710H, D712N, P714R, Y724H, E738K, F777S, or a combination thereof of the CTNNB1 polypeptide.

[0102] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *CTNNB1* DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *CTNNB1* DNA or RNA comprising one or more mutations within exon 3. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *CTNNB1* DNA or RNA comprising one or more mutations at codons 32, 33, 34, 37, 41, 45, 183, 245, 287 or a combination thereof. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *CTNNB1* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 25, 31, 32, 33, 34, 35, 36, 37, 41, 45, 140, 162, 170, 199, 213, 215, 257, 303, 322, 334, 354, 367, 373, 383, 387, 402, 426, 453, 474, 486, 515, 517, 535, 553, 555, 582, 587, 619, 623, 641, 646, 688, 703, 710, 712, 714, 724, 738, 777, or a combination thereof of the CTNNB1 polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *CTNNB1* DNA or RNA comprising one or more mutations selected from W25 (nonsense mutation), L31M, D32A, D32N, D32Y, D32G, D32H, S33C, S33Y, S33F, S33P, G34R, G34E, G34V, I35S, H36Y, S37F, S37P, S37C, S37A, T41N, T41A, T41I, S45Y, S45F, S45C, I140T, D162E, K170M, V199I, C213F, A215T, T257I, I303M, Q322K, E334K, K354T, G367V, P373S, W383G, N387K, L402F, N426D, R453L, R453Q, R474 (nonsense mutation), R486C, R515Q, L517F, R535 (nonsense mutation), R535Q, M553V, G555A, R582Q, R587Q, C619Y, Q623E, T641 (frame shift), S646F, M688T, Q703H, R710H, D712N, P714R, Y724H, E738K, F777S, or a combination thereof of the CTNNB1 polypeptide.

[0103] In some embodiments, beta-catenin associated genes further comprise *PIK3CA*, *PIK3CB*, and *MYC*. In some embodiments, beta-catenin associated genes further comprise *PIK3CA* DNA or RNA.

PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha or p110α protein) is a class i PI 3-kinase catalytic subunit that uses ATP to phosphorylate phosphatidylinositols. In some embodiments, *PIK3CA* gene is wild type *PIK3CA* or *PIK3CA* comprising one or more mutations. In some instances, *PIK3CA* DNA or RNA is wild type *PIK3CA*. In some instances, *PIK3CA* DNA or RNA comprises one or more mutations. In some instances, the polynucleic acid molecule hybridizes to a target region of wild type *PIK3CA* DNA or RNA. In some instances, the polynucleic acid molecule hybridizes to a target region of *PIK3CA* DNA or RNA comprising a mutation (e.g., a substitution, a deletion, or an addition).

[0104] In some embodiments, *PIK3CA* DNA or RNA comprisesone or more mutations. In some embodiments, *PIK3CA* DNA or RNA comprises one or more mutation within one or more exons. In some instances, *PIK3CA* DNA or RNA comprises one or more mutation within exons 9 and/or 20. In some instances, *PIK3CA* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 1, 4, 10-16, 11-18, 11, 12, 38, 39, 65, 72, 75, 79, 81, 83, 88, 90, 93, 102, 103, 103-104, 103-106, 104, 105-108, 106, 106-107, 106-108, 107, 108, 109-112, 110, 111, 113, 115, 137, 170, 258, 272, 279, 320, 328, 335, 342, 344, 345, 350, 357, 359, 363, 364, 365, 366, 378, 398, 401, 417, 420, 447-455, 449, 449-457, 451, 453, 454, 455, 455-460, 463-465, 471, 495, 522, 538, 539, 542, 545, 546, 547, 576, 604, 614, 617, 629, 643, 663, 682, 725, 726, 777, 791, 818, 866, 901, 909, 939, 951, 958, 970, 971, 975, 992, 1004, 1007, 1016, 1017, 1021, 1025, 1029, 1037, 1040, 1043, 1044, 1045, 1047, 1048, 1049, 1052, 1065, 1069, or a combination thereof of the PIK3CA polypeptide. In some embodiments, *PIK3CA* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues selected from M1V, R4 (nonsense mutation), L10-M16 (deletion), W11-P18 (deletion), W11L, G12D, R38L, R38H, R38C, R38S, E39K, E39G, E65K, S72G, Q75E, R79M, E81K, E81 (deletion), F83Y, R88Q, C90Y, C90G, R93Q, R93W, I102 (deletion), E103G, E103-P104 (deletion), E103-G106 (deletion), P104L, V105-R108 (deletion), G106V, G106-N107 (deletion), G106-R108 (deletion), G106R, N107S, R108L, R108H, E109-I112 (deletion), E110 (deletion), K111E, K111R, K111N, K111 (deletion), L113 (deletion), R115L, Q137L, N170S, D258N, Y272 (nonsense mutation), L279I, G320V, W328S, R335G, T342S, V344G, V344M, V344A, N345K, N345I, N345T, D350N, D350G, R357Q, G359R, G363A, G364R, E365K, E365V, P366R, C378R, C378Y, R398H, R401Q, E417K, C420R, C420G, P447-L455 (deletion), P449L, P449-N457 (deletion), G451R, G451V, E453K, E453Q, E453D, D454Y, L455 (frame shift insertion), L455-G460 (deletion), G463-N465 (deletion), P471L, P471A, H495L, H495Y, E522A, D538N, P539R, E542K, E542V, E542G, E542Q, E542A, E545K, E545A, E545G, E545Q, E545D, Q546K, Q546R, Q546P, E547D, S576Y, C604R, F614I, A617W, S629C, Q643H, I663S, Q682 (deletion), D725N, W726K, R777M, E791Q, R818C, L866W, C901F, F909L, D939G, R951C, Q958R, E970K, C971R, R975S, R992P, M1004I, G1007R, F1016C, D1017H, Y1021H, Y1021C, T1025A, T1025S, D1029H, E1037K, M1040V, M1043V, M1043I, N1044K, N1044Y, D1045V, H1047R, H1047L, H1047Y, H1047Q, H1048R, G1049R, T1052K, H1065L, 1069W (nonstop mutation), or a combination thereof of the PIK3CA polypeptide.

[0105] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CA* DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CA* DNA or RNA comprising one or more mutations within an exon. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CA* DNA or RNA comprising one or more mutations within exon 9 or exon 20. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CA* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 1, 4, 10-16, 11-18, 11, 12, 38, 39, 65, 72, 75, 79, 81, 83, 88, 90, 93, 102, 103, 103-104, 103-106, 104, 105-108, 106, 106-107, 106-108, 107, 108, 109-112, 110, 111, 113, 115, 137, 170, 258, 272, 279, 320, 328, 335, 342, 344, 345, 350, 357, 359, 363, 364, 365, 366, 378, 398, 401, 417, 420, 447-455, 449, 449-457, 451, 453, 454, 455, 455-460, 463-465, 471, 495, 522, 538, 539, 542, 545, 546, 547, 576, 604, 614, 617, 629, 643, 663, 682, 725, 726, 777, 791, 818, 866, 901, 909, 939, 951, 958, 970, 971, 975, 992, 1004, 1007, 1016, 1017, 1021, 1025, 1029, 1037, 1040, 1043, 1044, 1045, 1047, 1048, 1049, 1052, 1065, 1069, or a combination thereof of the PIK3CA polypeptide. In some embodiments, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of *PIK3CA* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues selected from M1V, R4 (nonsense mutation), L10-M16 (deletion), W11-P18 (deletion), W11L, G12D, R38L, R38H, R38C, R38S, E39K, E39G, E65K, S72G, Q75E, R79M, E81K, E81 (deletion), F83Y, R88Q, C90Y, C90G, R93Q, R93W, I102 (deletion), E103G, E103-P104 (deletion), E103-G106 (deletion), P104L, V105-R108 (deletion), G106V, G106-N107 (deletion), G106-R108 (deletion), G106R, N107S, R108L, R108H, E109-I112 (deletion), E110 (deletion), K111E, K111R, K111N, K111 (deletion), L113 (deletion), R115L, Q137L, N170S, D258N, Y272 (nonsense mutation), L279I, G320V, W328S, R335G, T342S, V344G, V344M, V344A, N345K, N345I, N345T, D350N, D350G, R357Q, G359R, G363A, G364R, E365K, E365V, P366R, C378R, C378Y, R398H, R401Q, E417K, C420R, C420G, P447-L455 (deletion), P449L, P449-N457 (deletion), G451R, G451V, E453K, E453Q, E453D, D454Y, L455 (frame shift insertion), L455-G460 (deletion), G463-N465 (deletion), P471L, P471A, H495L, H495Y, E522A, D538N, P539R, E542K, E542V, E542G, E542Q, E542A, E545K, E545A, E545G, E545Q, E545D, Q546K, Q546R, Q546P, E547D, S576Y, C604R, F614I, A617W, S629C, Q643H, I663S, Q682 (deletion), D725N, W726K, R777M, E791Q, R818C, L866W, C901F, F909L, D939G, R951C, Q958R, E970K, C971R, R975S, R992P, M1004I, G1007R, F1016C, D1017H, Y1021H, Y1021C, T1025A, T1025S, D1029H, E1037K, M1040V, M1043V, M1043I, N1044K, N1044Y, D1045V, H1047R, H1047L, H1047Y, H1047Q, H1048R, G1049R, T1052K, H1065L, 1069W (nonstop mutation), or a combination thereof of the PIK3CB polypeptide.

[0106] In some embodiments, beta-catenin associated genes further comprise *PIK3CB*. In some embodiments, *PIK3CB* gene is wild type or comprises one or more mutations. In some instances, *PIK3CB* DNA or RNA is wild type *PIK3CB* DNA or RNA. In some instances, *PIK3CB* DNA or RNA comprises one or more mutations. In some instances, the polynucleic acid molecule hybridizes to a target region of wild type *PIK3CB* DNA or RNA. In some instances, the polynucleic acid molecule hybridizes

to a target region of *PIK3CB* DNA or RNA comprising a mutation (e.g., a substitution, a deletion, or an addition).

[0107] In some embodiments, *PIK3CB* DNA or RNA comprises one or more mutations. In some embodiments, *PIK3CB* DNA or RNA comprises one or more mutations within one or more exons. In some instances, *PIK3CB* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 18, 19, 21, 28, 50, 61, 68, 103, 135, 140, 167, 252, 270, 290, 301, 304, 321, 369, 417, 442, 470, 497, 507, 512, 540, 551, 552, 554, 562, 567, 593, 595, 619, 628, 668, 768, 805, 824, 830, 887, 967, 992, 1005, 1020, 1036, 1046, 1047, 1048, 1049, 1051, 1055, 1067, or a combination thereof of the PIK3CB polypeptide. In some embodiments, *PIK3CB* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues selected from W18 (nonsense mutation), A19V, D21H, G28S, A50P, K61T, M68I, R103K, H135N, L140S, S167C, G252W, R270W, K290N, E301V, I304R, R321Q, V369I, T417M, N442K, E470K, E497D, P507S, I512M, E540 (nonsense mutation), C551R, E552K, E554K, R562 (nonsense mutation), E567D, A593V, L595P, V619A, R628 (nonsense mutation), R668W, L768F, K805E, D824E, A830T, E887 (nonsense mutation), V967A, I992T, A1005V, D1020H, E1036K, D1046N, E1047K, A1048V, L1049R, E1051K, T1055A, D1067V, D1067A, or a combination thereof of the PIK3CB polypeptide.

[0108] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CB* DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CB* DNA or RNA comprising one or more mutations within an exon. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CB* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 18, 19, 21, 28, 50, 61, 68, 103, 135, 140, 167, 252, 270, 290, 301, 304, 321, 369, 417, 442, 470, 497, 507, 512, 540, 551, 552, 554, 562, 567, 593, 595, 619, 628, 668, 768, 805, 824, 830, 887, 967, 992, 1005, 1020, 1036, 1046, 1047, 1048, 1049, 1051, 1055, 1067, or a combination thereof of the PIK3CB polypeptide.. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CB* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues selected from W18 (nonsense mutation), A19V, D21H, G28S, A50P, K61T, M68I, R103K, H135N, L140S, S167C, G252W, R270W, K290N, E301V, I304R, R321Q, V369I, T417M, N442K, E470K, E497D, P507S, I512M, E540 (nonsense mutation), C551R, E552K, E554K, R562 (nonsense mutation), E567D, A593V, L595P, V619A, R628 (nonsense mutation), R668W, L768F, K805E, D824E, A830T, E887 (nonsense mutation), V967A, I992T, A1005V, D1020H, E1036K, D1046N, E1047K, A1048V, L1049R, E1051K, T1055A, D1067V, D1067A, or a combination thereof of the PIK3CB polypeptide.

[0109] In some embodiments, beta-catenin associated genes further comprise *MYC*. In some embodiments, *MYC* gene is wild type *MYC* or *MYC* comprising one or more mutations. In some instances, *MYC* is wild type *MYC* DNA or RNA. In some instances, *MYC* DNA or RNA comprisesone or more mutations. In some instances, the polynucleic acid molecule hybridizes to a target region of wild type *MYC* DNA or RNA. In some instances, the polynucleic acid molecule is a polynucleic acid molecule

that hybridizes to a target region of *MYC* DNA or RNA comprising a mutation (e.g., a substitution, a deletion, or an addition).

[0110] In some embodiments, *MYC* DNA or RNA comprises one or more mutations. In some embodiments, MYC DNA or RNA comprises one or more mutation within one or more exons. In some instances, MYC DNA or RNA comprises one or more mutations within exon 2 or exon 3. In some instances, MYC DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 2, 7, 17, 20, 32, 44, 58, 59, 76, 115, 138, 141, 145, 146, 169, 175, 188, 200, 202, 203, 248, 251, 298, 321, 340, 369, 373, 374, 389, 395, 404, 419, 431, 439, or a combination thereof. In some embodiments, MYC DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues selected from P2L, F7L, D17N, Q20E, Y32N, A44V, A44T, T58I, P59L, A76V, F115L, F138S, A141S, V145I, S146L, S169C, S175N, C188F, N200S, S202N, S203T, T248S, D251E, S298Y, Q321E, V340D, V369D, T373K, H374R, F389L, Q395H, K404N, L419M, E431K, R439Q, or a combination thereof of the MYC polypeptide.

[0111] In some embodiments, the polynucleic acid molecule hybridizes to a target region of MYC DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to a target region of MYC DNA or RNA comprising one or more mutations within an exon. In some embodiments, the polynucleic acid molecule hybridizes to a target region of MYC DNA or RNA comprising one or more mutations within exon 2 or exon 3. In some embodiments, the polynucleic acid molecule hybridizes to a target region of MYC DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 2, 7, 17, 20, 32, 44, 58, 59, 76, 115, 138, 141, 145, 146, 169, 175, 188, 200, 202, 203, 248, 251, 298, 321, 340, 369, 373, 374, 389, 395, 404, 419, 431, 439, or a combination thereof of the MYC polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of MYC DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues selected from P2L, F7L, D17N, Q20E, Y32N, A44V, A44T, T58I, P59L, A76V, F115L, F138S, A141S, V145I, S146L, S169C, S175N, C188F, N200S, S202N, S203T, T248S, D251E, S298Y, Q321E, V340D, V369D, T373K, H374R, F389L, Q395H, K404N, L419M, E431K, R439Q, or a combination thereof of the MYC polypeptide.

Polynucleic Acid Molecules That Target Hypoxanthine Phosphoribosyltransferase 1 (HPRT1)

[0112] Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) is a transferase that catalyzes the conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate. HGPRT is encoded by the hypoxanthine Phosphoribosyltransferase 1 (*HPRT1*) gene.

[0113] In some embodiments, HPRT1 DNA or RNA is wild type or comprises one or more mutations. In some instances, *HPRT1* DNA or RNA comprises one or more mutations within one or more exons. In some instances, the one or more exons comprise exon 2, exon 3, exon 4, exon 6, exon 8, or exon 9. In some instances, HPRT1 DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 35, 48, 56, 74, 87, 129, 154, 162, 195, 200, 210, or a combination thereof of the HPRT1 polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region

of *HPRT1* DNA or RNA comprising one or more mutations selected from V35M, R48H, E56D, F74L, R87I, N129 (splice-site mutation), N154H, S162 (splice-site mutation), Y195C, Y195N, R200M, E210K, or a combination thereof of the HPRT1 polypeptide.

[0114] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *HPRT1* DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *HPRT1* DNA or RNA comprising one or more mutations within exon 2, exon 3, exon 4, exon 6, exon 8, or exon 9. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *HPRT1* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 35, 48, 56, 74, 87, 129, 154, 162, 195, 200, 210, or a combination thereof of the HPRT1 polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *HPRT1* DNA or RNA comprising one or more mutations selected from V35M, R48H, E56D, F74L, R87I, N129 (splice-site mutation), N154H, S162 (splice-site mutation), Y195C, Y195N, R200M, E210K, or a combination thereof of the HPRT1 polypeptide.

Polynucleic Acid Molecule Sequences

[0115] In some embodiments, the polynucleic acid molecule comprises a sequence that hybridizes to a target sequence illustrated in Tables 1, 3, 5, 6, or 7. In some instances, the polynucleic acid molecule is B. In some instances, the polynucleic acid molecule B comprises a sequence that hybridizes to a target sequence illustrated in Table 1 (KRAS target sequences). In some instances, the polynucleic acid molecule B comprises a sequence that hybridizes to a target sequence illustrated in Table 3 (EGFR target sequences). In some cases, the polynucleic acid molecule B comprises a sequence that hybridizes to a target sequence illustrated in Table 5 (AR target sequences). In some cases, the polynucleic acid molecule B comprises a sequence that hybridizes to a target sequence illustrated in Table 6 (β-catenin target sequences). In additional cases, the polynucleic acid molecule B comprises a sequence that hybridizes to a target sequence illustrated in Table 7 (PIK3CA and PIK3CB target sequences).

[0116] In some embodiments, the polynucleic acid molecule B 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 a sequence listed in Table 2. 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: 16-45. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 16-45. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 16-45. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 16-45. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 16-45. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 16-45. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 16-45. In some embodiments, the

polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 16-45. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 16-45. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 16-45. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 16-45. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 16-45. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 16-45. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 16-45.

[0117] In some embodiments, the polynucleic acid molecule B comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide 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: 16-45. In some cases, the second polynucleotide 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: 16-45. In some cases, the polynucleic acid molecule comprises a first polynucleotide 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: 16-45 and a second polynucleotide 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: 16-45.

[0118] In some embodiments, the polynucleic acid molecule B 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 a sequence listed in Table 4. 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: 422-1173. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 422-1173. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 422-1173. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 422-1173. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 422-1173. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 422-1173. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 422-1173. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 422-1173. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 422-1173. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 422-1173. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 422-1173. In some embodiments, the polynucleic acid molecule comprises a

sequence having at least 98% sequence identity to SEQ ID NOs: 422-1173. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 422-1173. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 422-1173.

[0119] In some embodiments, the polynucleic acid molecule B comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide 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: 422-1173. In some cases, the second polynucleotide 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: 422-1173. In some cases, the polynucleic acid molecule comprises a first polynucleotide 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: 422-1173 and a second polynucleotide 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: 422-1173.

[0120] In some embodiments, the polynucleic acid molecule B 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 a sequence listed in Table 8. 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: 1195-1214. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 1195-1214. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 1195-1214. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 1195-1214. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 1195-1214. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 1195-1214. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 1195-1214. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 1195-1214. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 1195-1214. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 1195-1214. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 1195-1214. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 1195-1214. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 1195-1214. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 1195-1214.

[0121] In some embodiments, the polynucleic acid molecule B comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide 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: 1195-1214. In some cases, the second polynucleotide comprises a sequence that is complementary to 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: 1195-1214. In some instances, the polynucleic acid molecule comprises a first polynucleotide 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: 1195-1214, and a second polynucleotide that is complementary to 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: 1195-1214.

[0122] In some embodiments, the polynucleic acid molecule B 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 a sequence listed in Table 9. 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: 1215-1242. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 1215-1242. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 1215-1242. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 1215-1242. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 1215-1242. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 1215-1242. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 1215-1242. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 1215-1242. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 1215-1242. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 1215-1242. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 1215-1242. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 1215-1242. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 1215-1242. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 1215-1242.

[0123] In some embodiments, the polynucleic acid molecule B comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide 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: 1215-1242. In some cases, the second polynucleotide 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: 1215-1242. In some cases, the polynucleic acid molecule comprises a

first polynucleotide 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: 1215-1242 and a second polynucleotide 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: 1215-1242.

Polynucleic Acid Molecules

[0124] In some embodiments, the polynucleic acid molecule described herein comprises RNA or DNA. In some cases, the polynucleic acid molecule 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 instances, the polynucleic acid molecule comprises siRNA. In some cases, B comprises siRNA.

[0125] In some embodiments, the polynucleic acid molecule is from about 10 to about 50 nucleotides in length. In some instances, the polynucleic acid molecule 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.

[0126] In some embodiments, the polynucleic acid molecule is about 50 nucleotides in length. In some instances, the polynucleic acid molecule is about 45 nucleotides in length. In some instances, the polynucleic acid molecule is about 40 nucleotides in length. In some instances, the polynucleic acid molecule is about 35 nucleotides in length. In some instances, the polynucleic acid molecule is about 30 nucleotides in length. In some instances, the polynucleic acid molecule is about 25 nucleotides in length. In some instances, the polynucleic acid molecule is about 20 nucleotides in length. In some instances, the polynucleic acid molecule is about 19 nucleotides in length. In some instances, the polynucleic acid molecule is about 18 nucleotides in length. In some instances, the polynucleic acid molecule is about 17 nucleotides in length. In some instances, the polynucleic acid molecule is about 16 nucleotides in length. In some instances, the polynucleic acid molecule is about 15 nucleotides in length. In some instances, the polynucleic acid molecule is about 14 nucleotides in length. In some instances, the polynucleic acid molecule is about 13 nucleotides in length. In some instances, the polynucleic acid molecule is about 12 nucleotides in length. In some instances, the polynucleic acid molecule is about 11 nucleotides in length. In some instances, the polynucleic acid molecule is about 10 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 50 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 45 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 40 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 35 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 30 nucleotides in length. In some instances, the

polynucleic acid molecule is from about 10 to about 25 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 20 nucleotides in length. In some instances, the polynucleic acid molecule is from about 15 to about 25 nucleotides in length. In some instances, the polynucleic acid molecule is from about 15 to about 30 nucleotides in length. In some instances, the polynucleic acid molecule is from about 12 to about 30 nucleotides in length.

[0127] In some embodiments, the polynucleic acid molecule comprises a first polynucleotide. In some instances, the polynucleic acid molecule comprises a second polynucleotide. In some instances, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide is a sense strand or passenger strand. In some instances, the second polynucleotide is an antisense strand or guide strand.

[0128] In some embodiments, the polynucleic acid molecule is a first polynucleotide. In some embodiments, the first polynucleotide is from about 10 to about 50 nucleotides in length. In some instances, the first polynucleotide 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.

[0129] In some instances, the first polynucleotide is about 50 nucleotides in length. In some instances, the first polynucleotide is about 45 nucleotides in length. In some instances, the first polynucleotide is about 40 nucleotides in length. In some instances, the first polynucleotide is about 35 nucleotides in length. In some instances, the first polynucleotide is about 30 nucleotides in length. In some instances, the first polynucleotide is about 25 nucleotides in length. In some instances, the first polynucleotide is about 20 nucleotides in length. In some instances, the first polynucleotide is about 19 nucleotides in length. In some instances, the first polynucleotide is about 18 nucleotides in length. In some instances, the first polynucleotide is about 17 nucleotides in length. In some instances, the first polynucleotide is about 16 nucleotides in length. In some instances, the first polynucleotide is about 15 nucleotides in length. In some instances, the first polynucleotide is about 14 nucleotides in length. In some instances, the first polynucleotide is about 13 nucleotides in length. In some instances, the first polynucleotide is about 12 nucleotides in length. In some instances, the first polynucleotide is about 11 nucleotides in length. In some instances, the first polynucleotide is about 10 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 50 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 45 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 40 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 35 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 30 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 25 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 20 nucleotides in length. In some instances, the first polynucleotide is from about 15 to about 25 nucleotides in length. In some instances, the first polynucleotide is from about 15 to about 30 nucleotides in length. In some instances, the first polynucleotide is from about 12 to about 30 nucleotides in length.

[0130] In some embodiments, the polynucleic acid molecule is a second polynucleotide. In some embodiments, the second polynucleotide is from about 10 to about 50 nucleotides in length. In some instances, the second polynucleotide 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.

[0131] In some instances, the second polynucleotide is about 50 nucleotides in length. In some instances, the second polynucleotide is about 45 nucleotides in length. In some instances, the second polynucleotide is about 40 nucleotides in length. In some instances, the second polynucleotide is about 35 nucleotides in length. In some instances, the second polynucleotide is about 30 nucleotides in length. In some instances, the second polynucleotide is about 25 nucleotides in length. In some instances, the second polynucleotide is about 20 nucleotides in length. In some instances, the second polynucleotide is about 19 nucleotides in length. In some instances, the second polynucleotide is about 18 nucleotides in length. In some instances, the second polynucleotide is about 17 nucleotides in length. In some instances, the second polynucleotide is about 16 nucleotides in length. In some instances, the second polynucleotide is about 15 nucleotides in length. In some instances, the second polynucleotide is about 14 nucleotides in length. In some instances, the second polynucleotide is about 13 nucleotides in length. In some instances, the second polynucleotide is about 12 nucleotides in length. In some instances, the second polynucleotide is about 11 nucleotides in length. In some instances, the second polynucleotide is about 10 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 50 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 45 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 40 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 35 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 30 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 25 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 20 nucleotides in length. In some instances, the second polynucleotide is from about 15 to about 25 nucleotides in length. In some instances, the second polynucleotide is from about 15 to about 30 nucleotides in length. In some instances, the second polynucleotide is from about 12 to about 30 nucleotides in length.

[0132] In some embodiments, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide. 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.

[0133] 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. 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 embodiments, the sequence of the polynucleic acid molecule is at least 99% 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.

[0134] 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 may has 3 or less mismatches to a target sequence described herein. In some cases, the sequence of the polynucleic acid molecule may has 2 or less mismatches to a target sequence described herein. In some cases, the sequence of the polynucleic acid molecule may has 1 or less mismatches to a target sequence described herein.

[0135] 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.

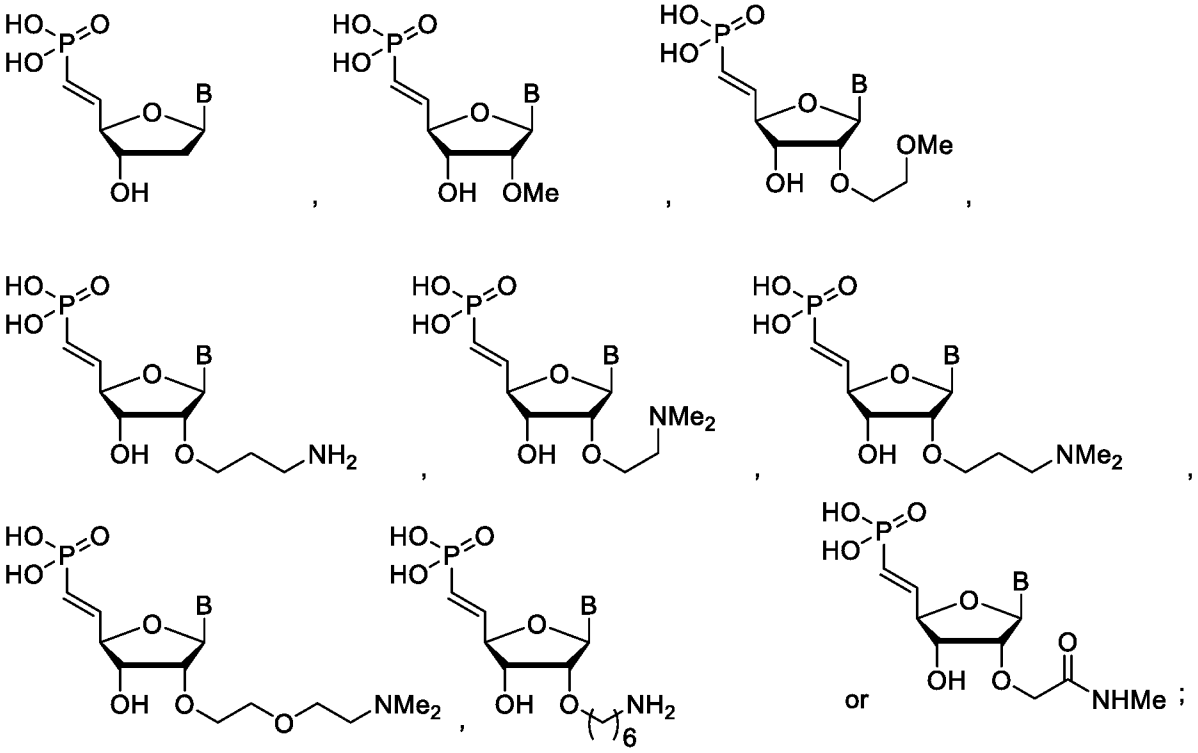
[0136] In some embodiments, the polynucleic acid molecule hybridizes to at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 8 contiguous bases of a target sequence 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.

[0137] 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.

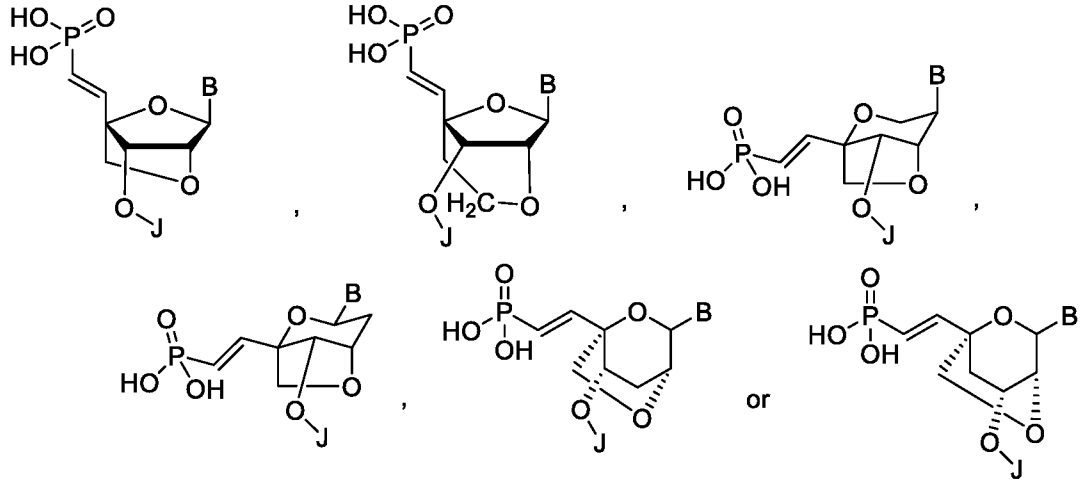
[0138] In some embodiments, the polynucleic acid molecule comprises natural, synthetic, or artificial nucleotide analogues or bases. In some cases, the polynucleic acid molecule comprises combinations of DNA, RNA and/or nucleotide analogues. In some instances, the synthetic or artificial nucleotide analogues or bases comprise modifications at one or more of ribose moiety, phosphate moiety, nucleoside moiety, or a combination thereof.

[0139] In some embodiments, a nucleotide analogue or artificial nucleotide base described above comprises a 5'-vinylphosphonate modified nucleotide nucleic acid with a modification at a 5' hydroxyl group of the ribose moiety. In some embodiments, the 5'-vinylphosphonate modified nucleotide is selected from the nucleotide provided below.



[0140] 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.

[0141] In some instances, the 5'-vinylphosphonate 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. Exemplary representations of the chemical structure of 5'-vinylphosphonate modified LNA are illustrated below, wherein J is an internucleotide linkage.

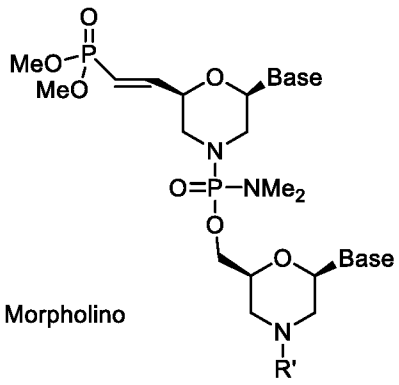


LNA (Locked Nucleic Acids)

[0142] In some embodiments, additional modifications at the 2' hydroxyl group include 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA).

[0143] 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, 2propylguanine, 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, carboxyalkylaminoalkyi nucleotides, and alkylcarbonylalkylated nucleotides. 5'-Vinylphosphonate modified nucleotides also include those nucleotides that are modified with respect to the sugar moiety, as well as 5'-vinylphosphonate 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.

[0144] In some embodiments, a 5'-vinylphosphonate modified nucleotide analogue further comprises a morpholino, a peptide nucleic acid (PNA), a methylphosphonate nucleotide, a thiolphosphonate 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'-vinylphosphonate modified morpholino oligonucleotide is illustrated below.

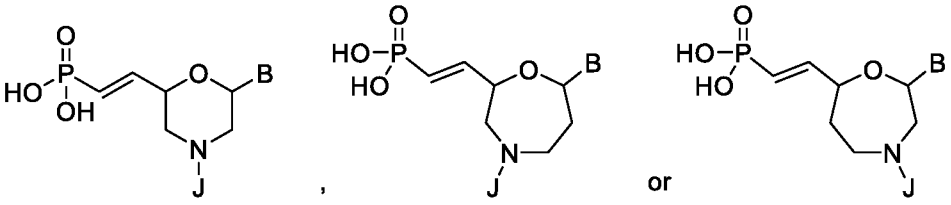


[0145] In some embodiments, a 5'-vinylphosphonate modified morpholino or PMO described above is a PMO comprising a positive or cationic charge. In some instances, the PMO is PMO*plus* (Sarepta). PMO*plus* refers to phosphorodiamidate morpholino oligomers comprising any number of (1-piperazino)phosphinylideneoxy, (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.

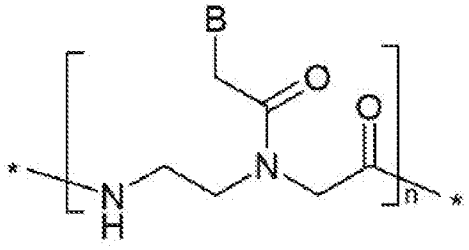
[0146] In some embodiments, a morpholino or PMO described above is a PMO-X (Sarepta). In some cases, PMO-X refers to phosphorodiamidate morpholino oligomers comprising at least one linkage or at least one of the disclosed terminal modifications, such as those disclosed in PCT Publication No. WO2011/150408 and U.S. Publication No. 2012/0065169.

[0147] In some embodiments, a morpholino or PMO described above is a PMO as described in Table 5 of U.S. Publication No. 2014/0296321.

[0148] Exemplary representations of the chemical structure of 5'-vinylphosphonate modified nucleic acids are illustrated below, wherein J is an internucleotide linkage.



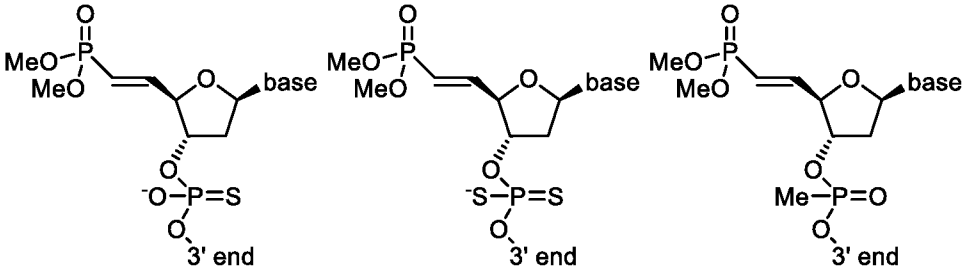
[0149] In some embodiments, peptide nucleic acid (PNA) does not contain sugar ring or phosphate linkage and the bases are attached and appropriately spaced by oligoglycine-like molecules, therefore, eliminating a backbone charge.



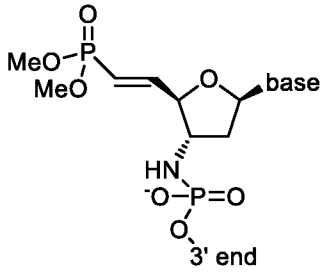
PNA

[0150] In some embodiments, one or more modifications of the 5'-vinylphosphonate modified oligonucleotide optionally 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.

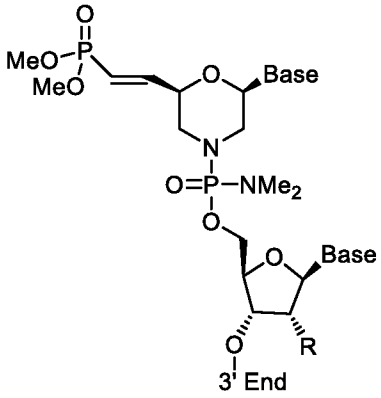
[0151] In some instances, the modification is a methyl or thiol modification such as methylphosphonate or thiolphosphonate modification. Exemplary thiolphosphonate nucleotide (left), phosphorodithioates (center) and methylphosphonate nucleotide (right) are illustrated below.



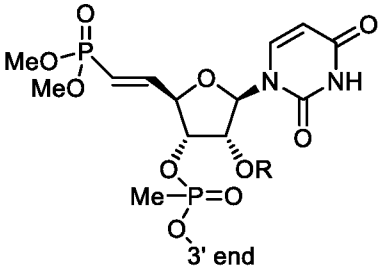
[0152] In some instances, a 5'-vinylphosphonate modified nucleotide includes, but is not limited to, phosphoramidites illustrated as:



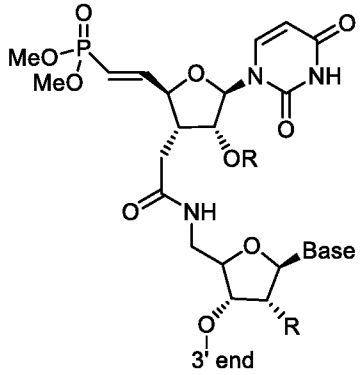
[0153] In some instances, the modified internucleotide linkage is a phosphorodiamidate linkage. A non-limiting example of a phosphorodiamidate linkage with a morpholino system is shown below.



[0154] In some instances, the modified internucleotide linkage is a methylphosphonate linkage. A non-limiting example of a methylphosphonate linkage is shown below.



[0155] In some instances, the modified internucleotide linkage is a amide linkage. A non-limiting example of an amide linkage is shown below.



[0156] In some instances, a 5'-vinylphosphonate modified nucleotide includes, but is not limited to, the modified nucleic acid illustrated illustrated below.

[0157] 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.

[0158] 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.

[0159] 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.

[0160] In some embodiments, the polynucleic acid molecule comprises one or more of the artificial nucleotide analogues described herein. In some instances, the 5'-vinylphosphonate modified polynucleic acid molecule 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 5'-vinylphosphonate modified nucleotide analogues described herein. In some embodiments, the artificial 5'-vinylphosphonate modified nucleotide analogues include 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (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. In some instances, the 5'-vinylphosphonate modified polynucleic acid molecule 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, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (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. In some instances, the 5'-vinylphosphonate modified polynucleic acid molecule 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 5'-vinylphosphonate modified polynucleic acid molecule 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 5'-vinylphosphonate modified polynucleic acid molecule 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.

[0161] In some embodiments, the 5'-vinylphosphonate modified polynucleic acid molecule 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, or more modifications. In some instances, the polynucleic acid molecule is a polynucleic acid molecule of SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242

[0162] In some instances, the 5'-vinylphosphonate modified polynucleic acid molecule 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, or more modified nucleotides. In some instances, the polynucleic acid molecule is a polynucleic acid molecule of SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242.

[0163] In some instances, the 5'-vinylphosphonate modified polynucleic acid molecule 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 some instances, the polynucleic acid molecule is a polynucleic acid molecule of SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242.

[0164] In some instances, about 5 to about 100% of the 5'-vinylphosphonate modified polynucleic acid molecule 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 instances, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of a polynucleic acid molecule of SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242 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 a polynucleic acid molecule of SEQ ID NOs: 16-45 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 a polynucleic acid molecule of SEQ ID NOs: 422-1173 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 a polynucleic acid molecule of SEQ ID NOs: 1195-1214 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 a polynucleic acid molecule of SEQ ID NOs: 1215-1242 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, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (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.

[0165] In some cases, one or more of the artificial 5'-vinylphosphonate modified nucleotide analogues described herein are resistant toward nucleases such as for example ribonuclease such as RNase H, deoxyribunuclease 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 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (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, deoxyribunuclease 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, T-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, T-O-dimethylaminoethyloxyethyl (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.

[0166] In some embodiments, one or more of the artificial 5'-vinylphosphonate modified nucleotide analogues 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 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-0-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (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, T-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, T-O-dimethylaminoethyloxyethyl (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, thiolphosphonate 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 Kd, a higher melt temperature (Tm), or a combination thereof.

[0167] In some embodiments, a 5'-vinylphosphonate modified polynucleic acid molecule 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.

[0168] 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.

[0169] 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, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-0-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (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.

[0170] 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. In some instances, the gene is *KRAS, EGFR, AR, HPRT1, CNNTB1* (β-catenin), or β-catenin associated genes.

[0171] 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).

[0172] One embodiment provides a molecule of Formula (I):

A-X-B-Y-C

Formula I

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or a first non-polymeric linker; and

Y is a bond or a second linker;

wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified non-natural nucleotide; and

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

[0173] Another embodiment provides the molecule of Formula (I), wherein the polynucleotide further comprises, at least one modified internucleotide linkage, or at least one inverted abasic moiety;

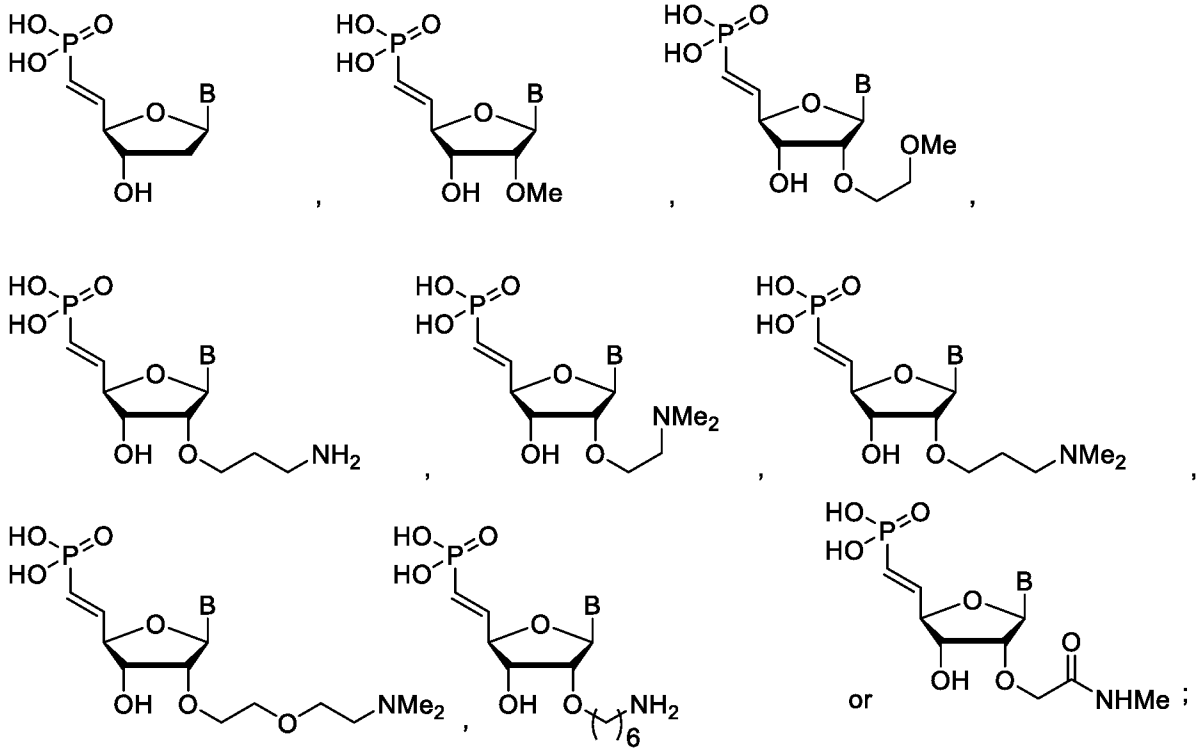
[0174] Another embodiment provides the molecule of Formula (I), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is located at the 5'-terminus of the polynucleotide.

[0175] Another embodiment provides the molecule of Formula (I), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is located at an internucleotide linkage of the polynucleotide.

[0176] Another embodiment provides the molecule of Formula (I), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is further modified at the 2'-position.

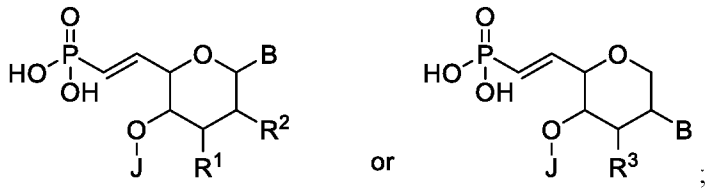
[0177] Another embodiment provides the molecule of Formula (I), wherein the 2'-modification is selected from 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide.

[0178] Another embodiment provides the molecule of Formula (I), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from:



wherein B is a heterocyclic base moiety.

[0179] Another embodiment provides the molecule of Formula (I), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from:

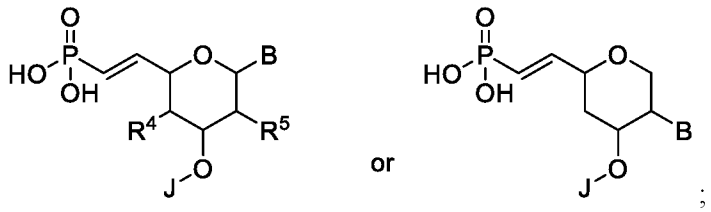


wherein B is a heterocyclic base moiety;

R1, R2, and R3 are independently selected from hydrogen, halogen, alkyl or alkoxy; and

J is an internucleotide linking group linking to the adjacent nucleotide of the polynucleotide.

[0180] Another embodiment provides the molecule of Formula (I), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from:

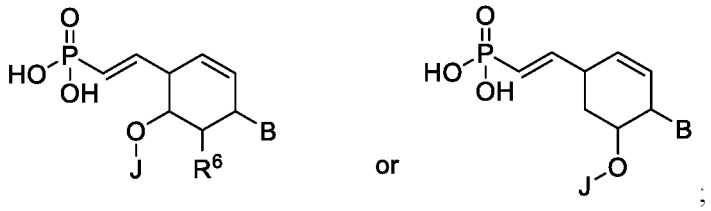


wherein B is a heterocyclic base moiety;

R4, and R5 are independently selected from hydrogen, halogen, alkyl or alkoxy; and

J is an internucleotide linking group linking to the adjacent nucleotide of the polynucleotide.

[0181] Another embodiment provides the molecule of Formula (I), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from:



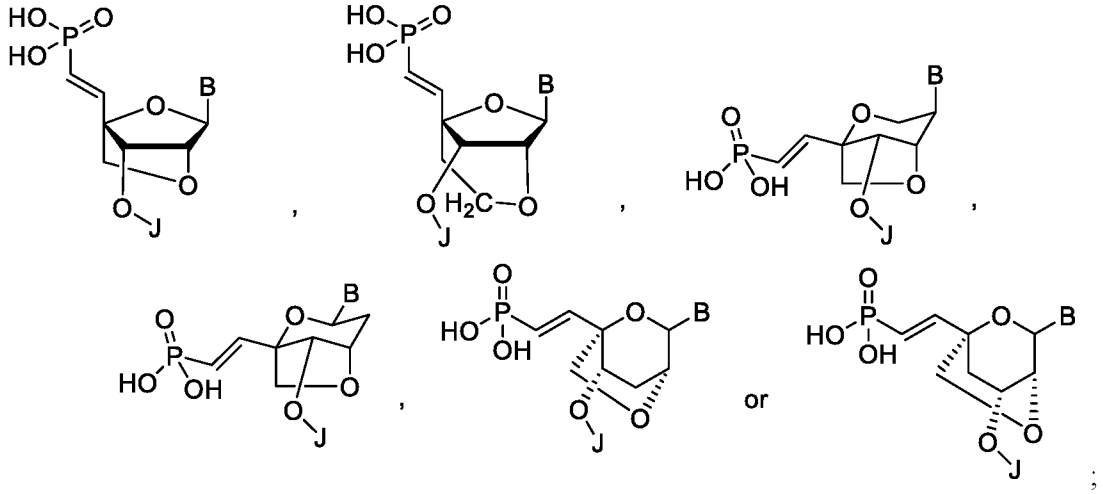
wherein B is a heterocyclic base moiety;

R6 is selected from hydrogen, halogen, alkyl or alkoxy; and

J is an internucleotide linking group linking to the adjacent nucleotide of the polynucleotide.

[0182] Another embodiment provides the molecule of Formula (I), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from locked nucleic acid (LNA) or ethylene nucleic acid (ENA).

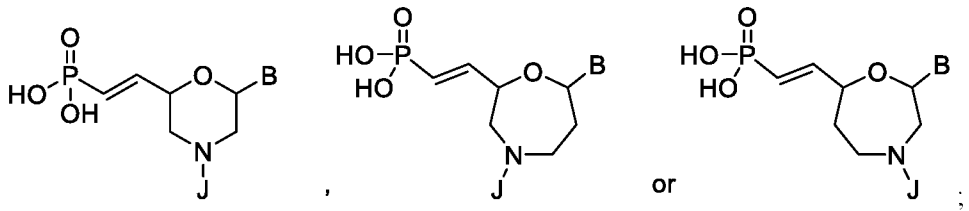
[0183] Another embodiment provides the molecule of Formula (I), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from:



wherein B is a heterocyclic base moiety; and

J is an internucleotide linking group linking to the adjacent nucleotide of the polynucleotide.

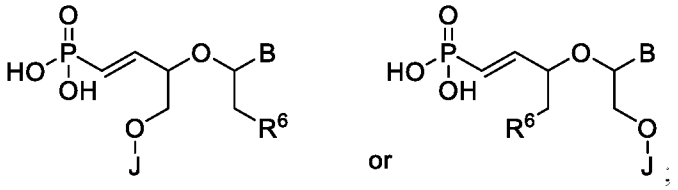
[0184] Another embodiment provides the molecule of Formula (I), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from:



wherein B is a heterocyclic base moiety; and

J is an internucleotide linking group linking to the adjacent nucleotide of the polynucleotide.

[0185] Another embodiment provides the molecule of Formula (I), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is:



wherein B is a heterocyclic base moiety;

R6 is selected from hydrogen, halogen, alkyl or alkoxy; and

J is an internucleotide linking group linking to the adjacent nucleotide of the polynucleotide.

[0186] Another embodiment provides the molecule of Formula (I), wherein the at least one modified internucleotide linkage comprises a phosphorothioate linkage, phosphorodithioate linkage, a phosphorodiamidate linkage, a methylphosphonate linkage, or an amide linkage.

[0187] Another embodiment provides the molecule of Formula (I), wherein the at least one inverted abasic moiety is at least one terminus.

[0188] One embodiment provides an oligonucleotide of Formula (II), wherein the oligonucleotide comprises at least one 5'-vinylphosphonate modified non-natural nucleotide.

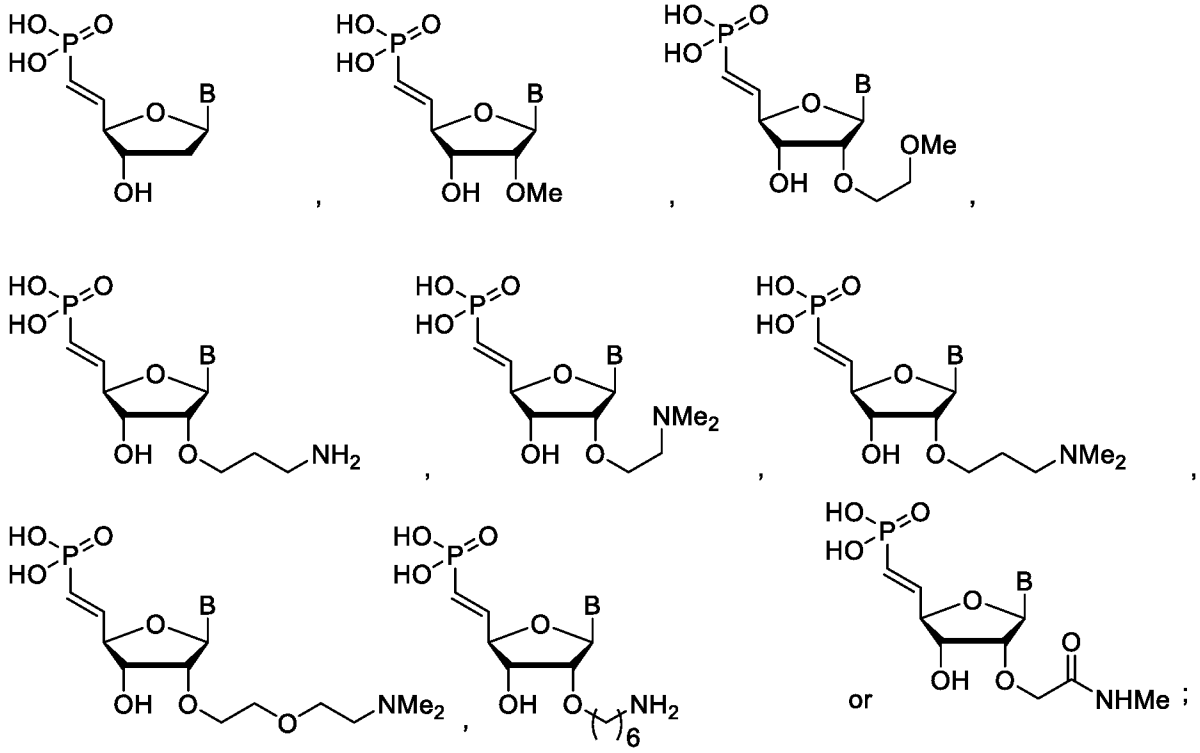
[0189] Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide further comprises, at least one modified internucleotide linkage, or at least one inverted abasic moiety;

[0190] Another embodiment provides the oligonucleotide of Formula (II), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is located at the 5'-terminus of the polynucleotide.

[0191] Another embodiment provides the oligonucleotide of Formula (II), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is located at an internucleotide linkage of the polynucleotide.

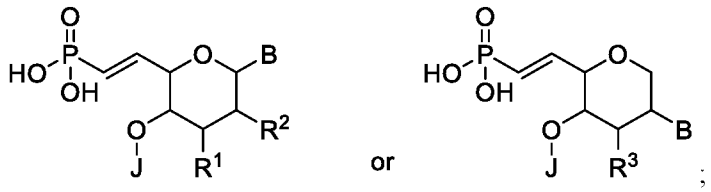
[0192] Another embodiment provides the oligonucleotide of Formula (II), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is further modified at the 2'-position. Another embodiment provides the oligonucleotide of Formula (II), wherein the 2'-modification is selected from 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide.

[0193] Another embodiment provides the oligonucleotide of Formula (II), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from:



wherein B is a heterocyclic base moiety.

[0194] Another embodiment provides the oligonucleotide of Formula (II), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from:

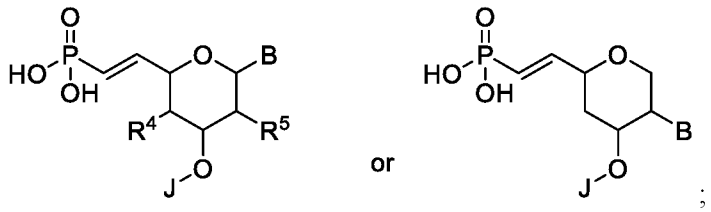


wherein B is a heterocyclic base moiety;

R1, R2, and R3 are independently selected from hydrogen, halogen, alkyl or alkoxy; and

J is an internucleotide linking group linking to the adjacent nucleotide of the polynucleotide.

[0195] Another embodiment provides the oligonucleotide of Formula (II), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from:

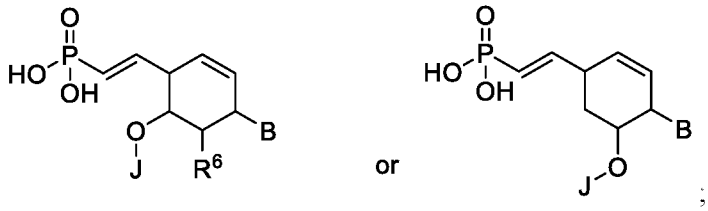


wherein B is a heterocyclic base moiety;

R4, and R5 are independently selected from hydrogen, halogen, alkyl or alkoxy; and

J is an internucleotide linking group linking to the adjacent nucleotide of the polynucleotide.

[0196] Another embodiment provides the oligonucleotide of Formula (II), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from:



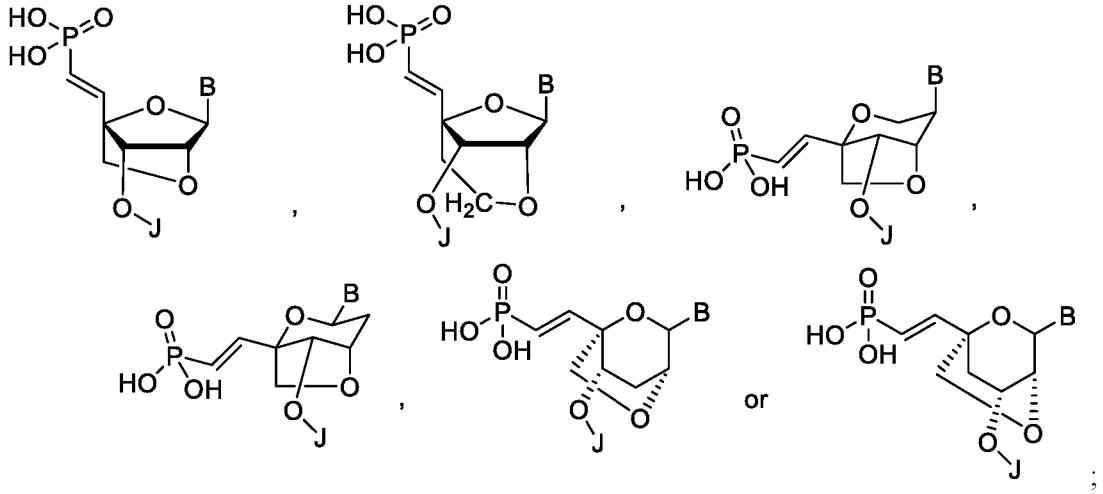
wherein B is a heterocyclic base moiety;

R6 is selected from hydrogen, halogen, alkyl or alkoxy; and

J is an internucleotide linking group linking to the adjacent nucleotide of the polynucleotide.

[0197] Another embodiment provides the oligonucleotide of Formula (II), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from locked nucleic acid (LNA) or ethylene nucleic acid (ENA).

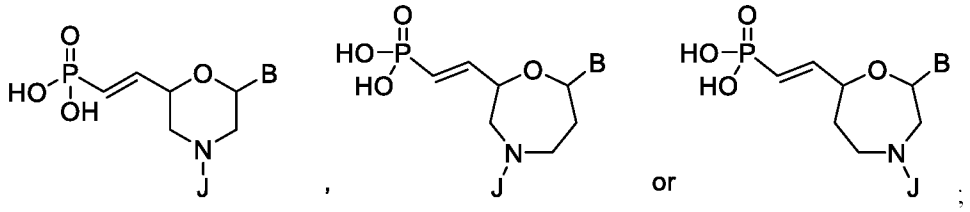
[0198] Another embodiment provides the oligonucleotide of Formula (II), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from:



wherein B is a heterocyclic base moiety; and

J is an internucleotide linking group linking to the adjacent nucleotide of the polynucleotide.

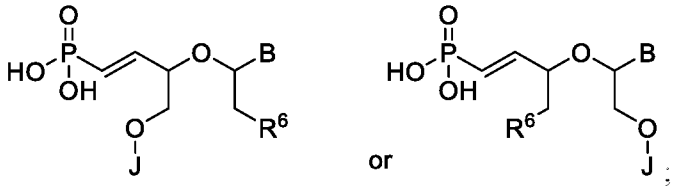
[0199] Another embodiment provides the oligonucleotide of Formula (II), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is selected from:



wherein B is a heterocyclic base moiety; and

J is an internucleotide linking group linking to the adjacent nucleotide of the polynucleotide.

[0200] Another embodiment provides the oligonucleotide of Formula (II), wherein the at least one 5'-vinylphosphonate modified non-natural nucleotide is:



wherein B is a heterocyclic base moiety;

R6 is selected from hydrogen, halogen, alkyl or alkoxy; and

J is an internucleotide linking group linking to the adjacent nucleotide of the polynucleotide.

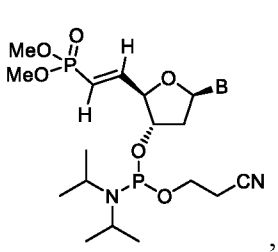
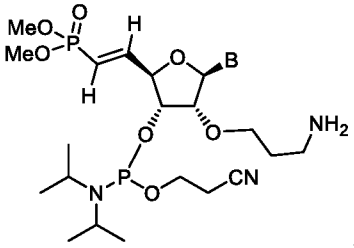
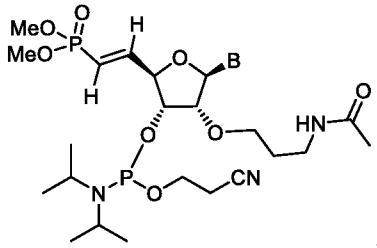
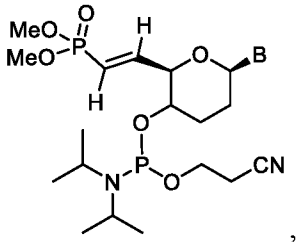
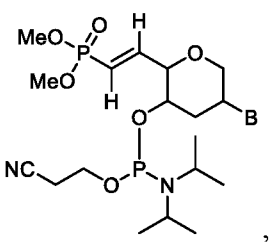
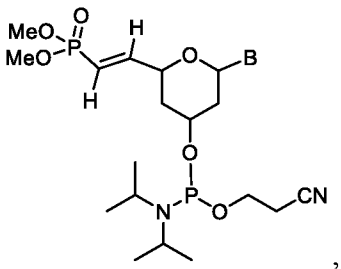
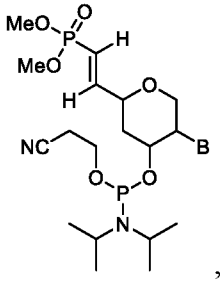
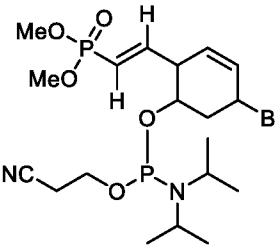
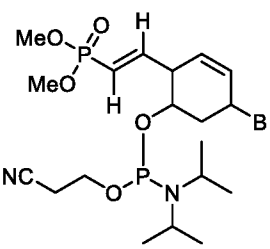
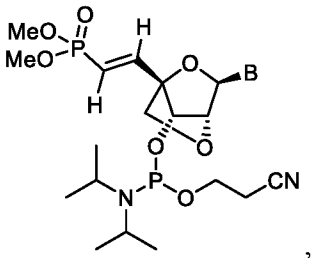
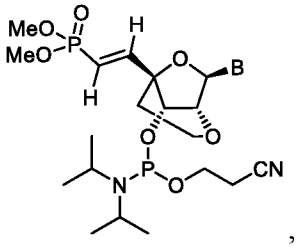
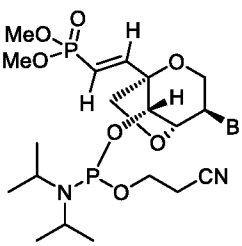
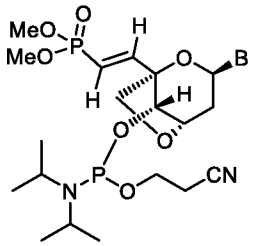
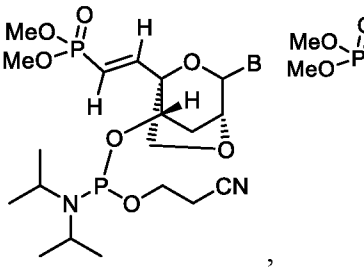
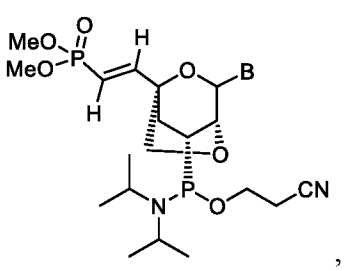
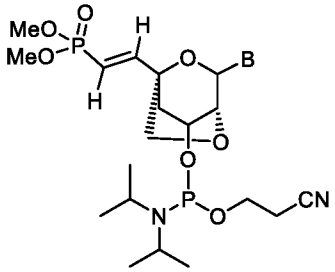
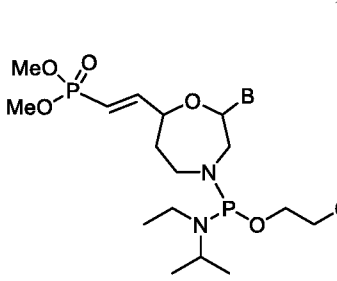
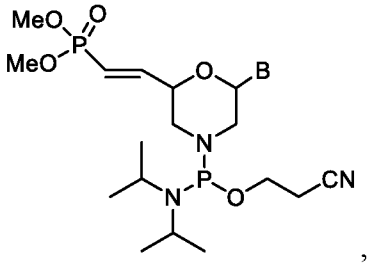
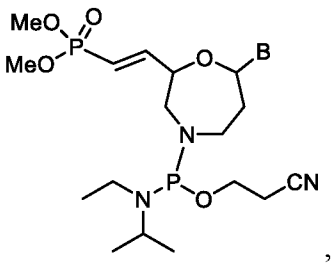
[0201] Another embodiment provides the oligonucleotide of Formula (II), wherein the at least one modified internucleotide linkage comprises a phosphorothioate linkage, phosphorodithioate linkage, a phosphorodiamidate linkage, a methylphosphonate linkage, or an amide linkage.

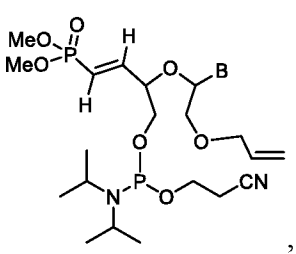
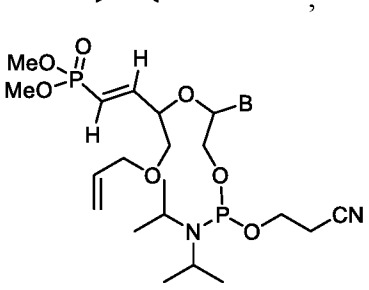
[0202] Another embodiment provides the oligonucleotide of Formula (II), wherein the at least one inverted abasic moiety is at least one terminus.

[0203] Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is single stranded. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is double stranded stranded.

[0204] Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 2 to about 100 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 2 to about 90 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 2 to about 80 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 2 to about 70 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 2 to about 60 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 2 to about 50 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 2 to about 40 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 2 to about 30 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 2 to about 20 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 2 to about 10 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 8 to about 30 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 10 to about 30 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 14 to about 30 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 18 to about 30 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 22 to about 30 residues in length. Another embodiment provides the oligonucleotide of Formula (II), wherein the oligonucleotide is from 26 to about 30 residues in length.

[0205] One embodiment provides a compound suitable for the synthesis of oligonucleotides selected from the group:


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wherein B is a heterocyclic base moiety.

Conjugation Chemistry

[0206] 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.

[0207] 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.

[0208] 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))

[0209] 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 derivatived 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)).

[0210] 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 alkylhydraine-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))

[0211] 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 transglutaminze 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))

[0212] 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.

[0213] 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

[0214] 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, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab_2, $F(ab)'_3$ 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 biding fragment thereof, or a chemically modified derivative thereof.

[0215] 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 Fab_2, $F(ab)'_3$ 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 biding 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 monovalent Fab'. In some instances, A is a diavalent Fab_2. In some instances, A is a single-chain variable fragment (scFv).

[0216] 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 FBTA05 (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 mAb^2 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 FBTA05 (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 mAb^2 from F-star Biotechnology Ltd.

[0217] In some cases, the bispecific antibody is a bispecific mini-antibody. In some instances, the bispecific mini-antibody comprises divalent Fab_2, $F(ab)'_3$ 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).

[0218] In some embodiments, the binding moiety A is a bispecific mini-antibody. In some instances, A is a bispecific Fab_2. 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).

[0219] 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).

[0220] 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, IL20Rα, 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), TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4) and the like.

[0221] 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, 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.

[0222] 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, IL20Rα, 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.

[0223] 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, 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.

[0224] 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 (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 (Blincyto, AMG103; Amgen), BEC2 (ImGlone Systems Inc.), carlumab (Janssen Biotech), catumaxomab (Removab, Trion Pharma), CEAcide (Immunomedics), Cetuximab (Erbitux®, 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, Aanofi-Aventis), futuximab, glaximab, ganitumab (Amgen), girentuximab (Rencarex®, Wilex 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), pemtumomab (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.

[0225] 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 (Blincyto, AMG103; Amgen), BEC2 (ImGlone Systems Inc.), carlumab (Janssen Biotech), catumaxomab (Removab, Trion Pharma), CEAcide (Immunomedics), Cetuximab (Erbitux®, 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, Aanofi-Aventis), futuximab, glaximab, ganitumab (Amgen), girentuximab (Rencarex®, Wilex 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), pemtumomab (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).

[0226] In some embodiments, the binding moiety A is conjugated according to Formula (I) to a polynucleic acid molecule (B), and a polymer (C), and optionally an endosomolytic moiety (D) according to Formula (II) described herein. In some instances, 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 a sequence listed in Tables 2, 4, 8, or 9. 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: 16-45, 422-1173, 1195-1214, or 1215-1242. In some instances, the polynucleic acid molecule comprises a sequence selected from SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242. In some instances, the polymer C comprises polyalkylen oxide (e.g., polyethylene glycol). In some embodiments, the endosomolytic moiety D comprises INF7 or melittin, or their respective derivatives.

[0227] In some embodiments, the binding moiety A is conjugated to a polynucleic acid molecule (B), and a polymer (C), and optionally an endosomolytic moiety (D). In some instances, the binding moiety A is an antibody or binding fragment thereof.

[0228] 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.

[0229] 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.

[0230] 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).

[0231] 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.

[0232] 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.

[0233] 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.

[0234] 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.

[0235] 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.

[0236] In some instances, an antibody binding fragment further encompasses its derivatives and includes polypeptide sequences containing at least one CDR.

[0237] 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.

[0238] 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.

[0239] 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.

[0240] 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.

Additional Binding Moieties

[0241] 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.

[0242] 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.

[0243] In some instances, the binding moiety is a polymer, including but not limited to poly nucleic 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.

[0244] 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).

[0245] 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).

Production of Antibodies or Binding Fragments Thereof

[0246] 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.

[0247] 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.

[0248] 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.

[0249] 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).

[0250] 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.

[0251] 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).

[0252] 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.

[0253] 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).

[0254] 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.

[0255] 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, 192, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes are employed in tk−, hgprt− or aprt− 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 hygro, 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).

[0256] 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).

[0257] 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

[0258] 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.

[0259] 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 polyalkylen oxide (e.g., polyethylene glycol). In some instances, the at least one polymer moiety C includes, but is not limited to, alpha-, omega-dihydroxylpolyethyleneglycol, biodegradable lactone-based polymer, e.g. polyacrylic acid, polylactide acid (PLA), poly(glycolic acid) (PGA), polypropylene, polystyrene, polyolefin, polyamide, polycyanoacrylate, polyimide, polyethylenterephthalat (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).

[0260] 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.

[0261] 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.

[0262] 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.

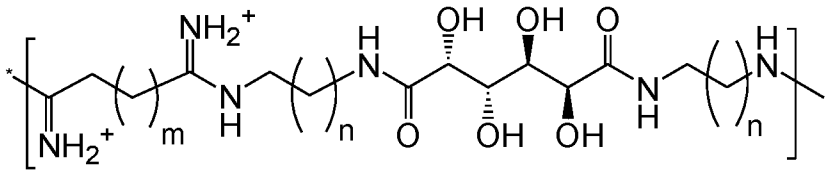
[0263] In some embodiments, C is polyalkylene oxide (e.g., PEG) and has a molecular weight of about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 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.

[0264] 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 instances, a dPEG comprises about 17 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 18 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 19 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 20 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 22 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 24 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 26 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 28 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 30 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 35 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 40 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 42 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 48 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 50 or more repeating ethylene oxide units. In some cases, a dPEG is synthesized as a single molecular weight compound from pure (e.g., about 95%, 98%, 99%, or 99.5%) staring material in a step-wise fashion. In some cases, a dPEG

has a specific molecular weight, rather than an average molecular weight. In some cases, a dPEG described herein is a dPEG from Quanta Biodesign, LMD.

[0265] 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):



Formula III

[0266] 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.

[0267] In some instances, cMAP is further conjugated to a PEG moiety, generating a cMAP-PEG copolymer, an mPEG-cMAP-PEGm triblock polymer, or a cMAP-PEG-cMAP triblock polymer. In some instances, the PEG moiety is in a range of from about 500 Da to about 50,000 Da. In some instances, the PEG moiety is in a range of from about 500 Da to about 1000 Da, greater than 1000 Da to about 5000 Da, greater than 5000 Da to about 10,000 Da, greater than 10,000 to about 25,000 Da, greater than 25,000 Da to about 50,000 Da,or any combination of two or more of these ranges.

[0268] 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.

[0269] In some embodiments, the polymer moiety C is conjugated to the polynucleic acid molecule, the binding moiety, and optionally to the endosomolytic moiety.

Endosomolytic Moiety

[0270] In some embodiments, a molecule of Formula (I): A-X-B-Y-C, further comprises an additional conjugating moiety. In some instances, the additional conjugating moiety is an endosomolytic 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.

Endosomolytic Polypeptides

[0271] In some embodiments, a molecule of Formula (I): A-X-B-Y-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.

[0272] In some instances, INF7 is a 24 residue polypeptide those sequence comprises CGIFGEIEELIEEGLENLIDWGNA (SEQ ID NO: 1243), or GLFEAIEGFIENGWEGMIDGWYGC (SEQ ID NO: 1244). In some instances, INF7 or its derivatives comprise a sequence of: GLFEAIEGFIENGWEGMIWDYGSGSCG (SEQ ID NO: 1245), GLFEAIEGFIENGWEGMIDG WYG-(PEG)6-NH2 (SEQ ID NO: 1246), or GLFEAIEGFIENGWEGMIWDYG-SGSC-K(GalNAc)2 (SEQ ID NO: 1247).

[0273] In some cases, melittin is a 26 residue polypeptide those sequence comprises CLIGAILKVLATGLPTLISWIKNKRKQ (SEQ ID NO: 1248), or GIGAVLKVLTTGLPALISWIKRKRQQ (SEQ ID NO: 1249). In some instances, melittin comprises a polypeptide sequence as described in U.S. Patent No. 8,501,930.

[0274] In some instances, meucin is an antimicrobial peptide (AMP) derived from the venom gland of the scorpion Mesobuthus eupeus. In some instances, meucin comprises of meucin-13 those sequence comprises IFGAIAGLLKNIF-NH_2 (SEQ ID NO: 1250) and meucin-18 those sequence comprises FFGHLFKLATKIIPSLFQ (SEQ ID NO: 1251).

[0275] In some instances, the endosomolytic polypeptide comprises a polypeptide in which its sequence is at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% sequence identity to INF7 or its derivatives thereof, melittin or its derivatives thereof, or meucin or its derivatives thereof. In some instances, the endosomolytic moiety comprises INF7 or its derivatives thereof, melittin or its derivatives thereof, or meucin or its derivatives thereof.

[0276] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide 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: 1243-1247. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1243. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1244-1247. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1243. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1244-1247. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1243. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1244-1247.

[0277] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide 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: 1248 or 1249. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1248. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1249. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1248. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1249. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1248. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1249.

[0278] In some instances, the endosomolytic moiety is meucin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide 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: 1250 or 1251. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1250. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1251. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1250. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1251. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1250. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1251.

[0279] In some instances, the endosomolytic moiety comprises a sequence as illustrated in Table 10.

Table 10.

Name	Origin	Amino Acid Sequence	SEQ ID NO:	Type
Pep-1	NLS from Simian Virus 40 large antigen and Reverse transcriptase of HIV	KETWWETWWTEWSQPKKKRKV	1252	Primary amphipathic
pVEC	VE-cadherin	LLIILRRRRIRKQAHAHSK	1253	Primary amphipathic
VT5	Synthetic peptide	DPKGDPKGVTVTVTVTVTGKGDPKPD	1254	β-sheet amphipathic
C105Y	1-antitrypsin	CSIPPEVKFNKPFVYLI	1255	-
Transportan	Galanin and mastoparan	GWTLNSAGYLLGKINLKALAALAKKIL	1256	Primary amphipathic
TP10	Galanin and mastoparan	AGYLLGKINLKALAALAKKIL	1257	Primary amphipathic
MPG	A hydrofobic domain from the fusion sequence of HIV gp41 and NLS of SV40 T antigen	GALFLGFLGAAGSTMGA	1258	β-sheet amphipathic
gH625	Glycoprotein gH of HSV type I	HGLASTLTRWAHYNALIRAF	1259	Secondary amphipathic

				α-helical
CADY	PPTG1 peptide	GLWRALWRLLRSLWRLLWRA	1260	Secondary amphipathic α-helical
GALA	Synthetic peptide	WEAALAEALAEALAEHLAEALAE ALEALAA	1261	Secondary amphipathic α-helical
INF	Influenza HA2 fusion peptide	GLFEAIEGFIENGWEGMIDGWYGC	1262	Secondary amphipathic α-helical/ pH-dependent membrane active peptide
HA2E5-TAT	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAGFIENGWEGMIDGWYG	1263	Secondary amphipathic α-helical/ pH-dependent membrane active peptide
HA2-penetratin	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAGFIENGWEGMIDGRQIKI WFQNRRMKW KK-amide	1264	pH-dependent membrane active peptide
HA-K4	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAGFIENGWEGMIDG-SSKKKK	1265	pH-dependent membrane active peptide
HA2E4	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFEAIAGFIENGWEGMIDGGGYC	1266	pH-dependent membrane active peptide
H5WYG	HA2 analogue	GLFHAIAHFIHGGWH GLIHGWYG	1267	pH-dependent membrane active peptide
GALA-INF3-(PEG)6-NH	INF3 fusion peptide	GLFEAIEGFIENGWEGLAEALAEAL EALAA-(PEG)6-NH2	1268	pH-dependent membrane active peptide
CM18-TAT11	Cecropin-A-Melittin$_{2-12}$ (CM_{18}) fusion peptide	KWKLFKKIGAVLKVLTTG-YGRKKRRQRRR	1269	pH-dependent membrane active peptide

[0280] 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).

[0281] 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 Polymers

[0282] In some embodiments, a molecule of Formula (I): A-X-B-Y-C, is further conjugated with an endosomolytic polymer. As used herein, an endosomolytic polymer comprises a linear, a branched network, a star, a comb, or a ladder type of polymer. In some instances, an endosomolytic polymer is a homopolymer or a copolymer comprising two ro more different types of monomers. In some cases, an endosomolytic polymer is a polycation polymer. In other cases, an endosomolytic polymer is a polyanion polymer.

[0283] In some instances, a polycation polymer comprises monomer units that are charge positive, charge neutral, or charge negative, with a net charge being positive. In other cases, a polycation polymer comprises a non-polymeric molecule that contains two or more positive charges. Exemplary cationic polymers include, but are not limited to, poly(L-lysine) (PLL), poly(L-arginine) (PLA), polyethyleneimine (PEI), poly[α-(4-aminobutyl)-L-glycolic acid] (PAGA), 2-(dimethylamino)ethyl methacrylate (DMAEMA), or N,N-Diethylaminoethyl Methacrylate (DEAEMA).

[0284] In some cases, a polyanion polymer comprises monomer units that are charge positive, charge neutral, or charge negative, with a net charge being negative. In other cases, a polyanion polymer comprises a non-polymeric molecule that contains two or more negative charges. Exemplary anionic polymers include p(alkylacrylates) (e.g., poly(propyl acrylic acid) (PPAA)) or poly(N-isopropylacrylamide) (NIPAM). Additional examples include PP75, a L-phenylalanine-poly(L-lysine isophthalamide) polymer described in Khormaee, *et al*., "Edosomolytic anionic polymer for the cytoplasmic delivery of siRNAs in localized in vivo applications," *Advanced Functional Materials* **23**: 565-574 (2013).

[0285] In some embodiments, an endosomolytic polymer described herein is a pH-responsive endosomolytic polymer. A pH-responsive polymer comprises a polymer that increases in size (swell) or collapses depending on the pH of the environment. Polyacrylic acid and chitosan are examples of pH-responsive polymers.

[0286] In some instances, an endosomolytic moiety described herein is a membrane-disruptive polymer. In some cases, the membrane-disruptive polymer comprises a cationic polymer, a neutral or hydrophobic polymer, or an anionic polymer. In some instances, the membrane-disruptive polymer is a hydrophilic polymer.

[0287] In some instances, an endosomolytic moiety described herein is a pH-responsive membrane-disruptive polymer. Exemplary pH-responsive membrane-disruptive polymers include p(alkylacrylic acids), poly(N-isopropylacrylamide) (NIPAM) copolymers, succinylated p(glycidols), and p(β-malic acid) polymers.

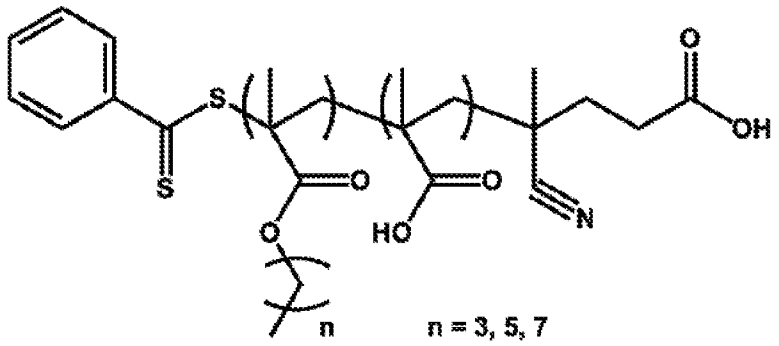
[0288] In some instances, p(alkylacrylic acids) include poly(propylacrylic acid) (polyPAA), poly(methacrylic acid) (PMAA), poly(ethylacrylic acid) (PEAA), and poly(propyl acrylic acid) (PPAA). In some instances, a p(alkylacrylic acid) include a p(alkylacrylic acid) described in Jones, *et al.*, *Biochemistry Journal* **372**: 65-75 (2003).

[0289] In some embodiments, a pH-responsive membrane-disruptive polymer comprises p(butyl acrylate-co-methacrylic acid). (*see* Bulmus, *et al.*, *Journal of Controlled Release* **93**: 105-120 (2003); and Yessine, *et al.*, *Biochimica et Biophysica Acta* **1613**: 28-38 (2003))

[0290] In some embodiments, a pH-responsive membrane-disruptive polymer comprises p(styrene-alt-maleic anhydride). (*see* Henry, *et al.*, *Biomacromolecules* 7: 2407-2414 (2006))

[0291] In some embodiments, a pH-responsive membrane-disruptive polymer comprises pyridyldisulfide acrylate (PDSA) polymers such as poly(MAA-*co*-PDSA), poly(EAA-*co*-PDSA), poly(PAA-*co*-PDSA), poly(MAA-*co*-BA-*co*-PDSA), poly(EAA-*co*-BA-*co*-PDSA), or poly(PAA-*co*-BA-*co*-PDSA) polymers. (*see* El-Sayed, *et al.*, "Rational design of composition and activity correlations for pH-responsive and glutathione-reactive polymer therapeutics," *Journal of Controlled Release* **104**: 417-427 (2005); or Flanary *et al.*, "Antigen delivery with poly(propylacrylic acid) conjugation enhanced MHC-1 presentation and T-cell activation," *Bioconjugate Chem.* **20**: 241-248 (2009))

[0292] In some embodiments, a pH-responsive membrane-disruptive polymer comprises a lytic polymer comprising the base structure of:



[0293] In some instances, an endosomolytic moiety described herein is further conjugated to an additional conjugate, e.g., a polymer (e.g., PEG), or a modified polymer (e.g., cholesterol-modified polymer).

[0294] In some instances, the additional conjugate comprises a detergent (e.g., Triton X-100). In some instances, an endosomolytic moiety described herein comprises a polymer (e.g., a poly(amidoamine)) conjugated with a detergent (e.g., Triton X-100). In some instances, an endosomolytic moiety described herein comprises poly(amidoamine)-Triton X-100 conjugate (Duncan, *et al.*, "A polymer-Triton X-100 conjugate capable of pH-dependent red blood cell lysis: a model system

illustrating the possibility of drug delivery within acidic intracellular compartments," *Journal of Drug Targeting* **2**: 341-347 (1994)).

Endosomolytic Lipids

[0295] In some embodiments, the endosomolytic moiety is a lipid (e.g., a fusogenic lipid). In some embodiments, a molecule of Formula (I): A-X-B-Y-C, is further conjugated with an endosomolytic lipid (e.g., fusogenic lipid). Exemplary fusogenic lipids include 1,2-dileoyl-sn-3-phosphoethanolamine (DOPE), phosphatidylethanolamine (POPE), palmitoyloleoylphosphatidylcholine (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).

[0296] 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

[0297] In some embodiments, the endosomolytic moiety is a small molecule. In some embodiments, a molecule of Formula (I): A-X-B-Y-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.

Formula (I) Molecule -Endosomolytic Moiety Conjugates

[0298] In some embodiments, one or more endosomolytic moieties are conjugated to a molecule comprising at least one binding moiety, at least one polynucleotide, at least one polymer, or any combinations thereof. In some instances, the endosomolytic moiety is conjugated according to Formula (II):

$$(A\text{-}X\text{-}B\text{-}Y\text{-}C_c)\text{-}L\text{-}D$$

Formula II

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or a first linker;

Y is a bond or a second linker;

L is a bond or a third linker;

D is an endosomolytic moiety; and

c is an integer between 0 and 1; and

wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide; and D is conjugated anywhere on A, B, or C.

[0299] In some embodiments, A and C are not attached to B at the same terminus.

[0300] In some embodiments, the at least one 2' modified nucleotide comprises 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some instances, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some cases, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In some instances, the second polynucleotide comprises at least one modification. In some cases, the first polynucleotide and the second polynucleotide are RNA molecules. In some cases, the first polynucleotide and the second polynucleotide are siRNA molecules. In some embodiments, X, Y, and L are independently a bond or a non-polymeric linker group. In some instances, A is an antibody or binding fragment thereof. In some instances, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, 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. In some cases, C is polyethylene glycol.

[0301] In some instances, the endosomolytic moiety comprises a polypeptide, a polymer, a lipid, or a small molecule. In some instances, the endosomolytic moiety is an endosomolytic polypeptide. In some cases, the endosomolytic moiety is an endosomolytic polymer. In other cases, the endosomolytic moiety is an endosomolytic lipid. In additional cases, the endosomolytic moiety is an endosomolytic small molecule.

[0302] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide 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: 1243. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1243. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1243.

[0303] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide 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: 1248. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1248. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1248.

[0304] In some instances, the endosomolytic moiety is a sequence as illustrated in Table 10.

[0305] In additional cases, the endosomolytic moiety is an endosomolytic polymer, such as for example, a pH-responsive endosomolytic polymer, a membrane-disruptive polymer, a polycation polymer, a polyanion polymer, a pH-responsive membrane-disruptive polymer, or a combination thereof. In additional cases, the endosomolytic moiety comprises a p(alkylacrylic acid) polymer, a p(butyl

acrylate-co-methacrylic acid) polymer, a p(styrene-alt-maleic anhydride) polymer, a pyridyldisulfide acrylate (PDSA) polymer, a polymer-PEG conjugate, a polymer-detergent conjugate, or a combination thereof.

[0306] In some embodiments, the endosomolytic moiety conjugate is according to Formula (IIa):

$$D\text{-}L\text{-}A\text{-}X\text{-}B\text{-}Y\text{-}C_c$$

Formula IIa

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or a first linker;

Y is a bond or a second linker;

L is a bond or a third linker;

D is an endosomolytic moiety; and

c is an integer of 1; and

wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide.

[0307] In some embodiments, A and C are not attached to B at the same terminus.

[0308] In some embodiments, the at least one 2' modified nucleotide comprises 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some instances, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some cases, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In some instances, the second polynucleotide comprises at least one modification. In some cases, the first polynucleotide and the second polynucleotide are RNA molecules. In some cases, the first polynucleotide and the second polynucleotide are siRNA molecules. In some embodiments, X, Y, and L are independently a bond or a non-polymeric linker group. In some instances, A is an antibody or binding fragment thereof. In some instances, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, 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. In some cases, C is polyethylene glycol.

[0309] In some instances, the endosomolytic moiety comprises a polypeptide, a polymer, a lipid, or a small molecule. In some instances, the endosomolytic moiety is an endosomolytic polypeptide. In some cases, the endosomolytic moiety is an endosomolytic polymer. In other cases, the endosomolytic moiety

is an endosomolytic lipid. In additional cases, the endosomolytic moiety is an endosomolytic small molecule.

[0310] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide 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: 1243. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1243. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1243.

[0311] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide 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: 1248. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1248. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1248.

[0312] In some instances, the endosomolytic moiety is a sequence as illustrated in Table 10.

[0313] In additional cases, the endosomolytic moiety is an endosomolytic polymer, such as for example, a pH-responsive endosomolytic polymer, a membrane-disruptive polymer, a polycation polymer, a polyanion polymer, a pH-responsive membrane-disruptive polymer, or a combination thereof. In additional cases, the endosomolytic moiety comprises a p(alkylacrylic acid) polymer, a p(butyl acrylate-co-methacrylic acid) polymer, a p(styrene-alt-maleic anhydride) polymer, a pyridyldisulfide acrylate (PDSA) polymer, a polymer-PEG conjugate, a polymer-detergent conjugate, or a combination thereof.

[0314] In some instances, the endosomolytic moiety conjugate is according to Formula (IIb):

A-X-B-L-D

Formula IIb

wherein,

A is a binding moiety;

B is a polynucleotide;

X is a bond or a first linker;

L is a bond or a third linker; and

D is an endosomolytic moiety; and

wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide.

[0315] In some embodiments, A and C are not attached to B at the same terminus.

[0316] In some embodiments, the at least one 2' modified nucleotide comprises 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some instances, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some cases, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the

polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In some instances, the second polynucleotide comprises at least one modification. In some cases, the first polynucleotide and the second polynucleotide are RNA molecules. In some cases, the first polynucleotide and the second polynucleotide are siRNA molecules. In some embodiments, X and L are independently a bond or a non-polymeric linker group. In some instances, A is an antibody or binding fragment thereof. In some instances, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, 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. In some cases, C is polyethylene glycol.

[0317] In some instances, the endosomolytic moiety comprises a polypeptide, a polymer, a lipid, or a small molecule. In some instances, the endosomolytic moiety is an endosomolytic polypeptide. In some cases, the endosomolytic moiety is an endosomolytic polymer. In other cases, the endosomolytic moiety is an endosomolytic lipid. In additional cases, the endosomolytic moiety is an endosomolytic small molecule.

[0318] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide 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: 1243. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1243. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1243.

[0319] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide 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: 1248. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1248. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1248.

[0320] In some instances, the endosomolytic moiety is a sequence as illustrated in Table 10.

[0321] In additional cases, the endosomolytic moiety is an endosomolytic polymer, such as for example, a pH-responsive endosomolytic polymer, a membrane-disruptive polymer, a polycation polymer, a polyanion polymer, a pH-responsive membrane-disruptive polymer, or a combination thereof. In additional cases, the endosomolytic moiety comprises a p(alkylacrylic acid) polymer, a p(butyl acrylate-co-methacrylic acid) polymer, a p(styrene-alt-maleic anhydride) polymer, a pyridyldisulfide acrylate (PDSA) polymer, a polymer-PEG conjugate, a polymer-detergent conjugate, or a combination thereof.

[0322] In some instances, the endosomolytic moiety conjugate is according to Formula (IIc):

$$A\text{-}X\text{-}B\text{-}Y\text{-}C_c\text{-}L\text{-}D$$

Formula IIc

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or a first linker;

Y is a bond or a second linker;

L is a bond or a third linker;

D is an endosomolytic moiety; and

c is an integer of 1; and

wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide.

[0323] In some embodiments, A and C are not attached to B at the same terminus.

[0324] In some embodiments, the at least one 2' modified nucleotide comprises 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some instances, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some cases, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In some instances, the second polynucleotide comprises at least one modification. In some cases, the first polynucleotide and the second polynucleotide are RNA molecules. In some cases, the first polynucleotide and the second polynucleotide are siRNA molecules. In some embodiments, X, Y, and L are independently a bond or a non-polymeric linker group. In some instances, A is an antibody or binding fragment thereof. In some instances, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, 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. In some cases, C is polyethylene glycol.

[0325] In some instances, the endosomolytic moiety comprises a polypeptide, a polymer, a lipid, or a small molecule. In some instances, the endosomolytic moiety is an endosomolytic polypeptide. In some cases, the endosomolytic moiety is an endosomolytic polymer. In other cases, the endosomolytic moiety is an endosomolytic lipid. In additional cases, the endosomolytic moiety is an endosomolytic small molecule.

[0326] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide 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: 1243. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1243. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1243.

[0327] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide 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: 1248. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1248. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1248.

[0328] In some instances, the endosomolytic moiety is a sequence as illustrated in Table 10.

[0329] In additional cases, the endosomolytic moiety is an endosomolytic polymer, such as for example, a pH-responsive endosomolytic polymer, a membrane-disruptive polymer, a polycation polymer, a polyanion polymer, a pH-responsive membrane-disruptive polymer, or a combination thereof. In additional cases, the endosomolytic moiety comprises a p(alkylacrylic acid) polymer, a p(butyl acrylate-co-methacrylic acid) polymer, a p(styrene-alt-maleic anhydride) polymer, a pyridyldisulfide acrylate (PDSA) polymer, a polymer-PEG conjugate, a polymer-detergent conjugate, or a combination thereof.

[0330] In some instances, the endosomolytic moiety conjugate is according to Formula (IId):

$$A\text{-}L\text{-}D\text{-}X\text{-}B\text{-}Y\text{-}C_c$$

Formula IId

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or a first linker;

Y is a bond or a second linker;

L is a bond or a third linker;

D is an endosomolytic moiety; and

c is an integer of 1; and

wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide.

[0331] In some embodiments, A and C are not attached to B at the same terminus.

[0332] In some embodiments, the at least one 2' modified nucleotide comprises 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some instances, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some cases, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In some instances, the second polynucleotide comprises at least one modification. In some cases, the first polynucleotide and the second polynucleotide are RNA molecules. In some cases, the first polynucleotide and the second

polynucleotide are siRNA molecules. In some embodiments, X, Y, and L are independently a bond or a non-polymeric linker group. In some instances, A is an antibody or binding fragment thereof. In some instances, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, 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. In some cases, C is polyethylene glycol.

[0333] In some instances, the endosomolytic moiety comprises a polypeptide, a polymer, a lipid, or a small molecule. In some instances, the endosomolytic moiety is an endosomolytic polypeptide. In some cases, the endosomolytic moiety is an endosomolytic polymer. In other cases, the endosomolytic moiety is an endosomolytic lipid. In additional cases, the endosomolytic moiety is an endosomolytic small molecule.

[0334] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide 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: 1243. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1243. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1243.

[0335] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide 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: 1248. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1248. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1248.

[0336] In some instances, the endosomolytic moiety is a sequence as illustrated in Table 10.

[0337] In additional cases, the endosomolytic moiety is an endosomolytic polymer, such as for example, a pH-responsive endosomolytic polymer, a membrane-disruptive polymer, a polycation polymer, a polyanion polymer, a pH-responsive membrane-disruptive polymer, or a combination thereof. In additional cases, the endosomolytic moiety comprises a p(alkylacrylic acid) polymer, a p(butyl acrylate-co-methacrylic acid) polymer, a p(styrene-alt-maleic anhydride) polymer, a pyridyldisulfide acrylate (PDSA) polymer, a polymer-PEG conjugate, a polymer-detergent conjugate, or a combination thereof.

Linkers

[0338] 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 C_1-C_6 alkyl group (e.g., a C_5, C_4, C_3, C_2, or C_1 alkyl group). In some instances, the linker includes homobifunctional cross linkers, heterobifunctional cross linkers, and the like. In some instances, the liker

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.

[0339] In some instances, the linker comprises a homobifuctional linker. Exemplary homobifuctional linkers include, but are not limited to, Lomant's reagent dithiobis (succinimidylpropionate) DSP, 3′3′-dithiobis(sulfosuccinimidyl proprionate (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), bismaleimidohexane (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, carbohydrazide, 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).

[0340] 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), succinimidyloxycarbonyl-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-iodoacteyl)aminobenzoate (sIAB), sulfosuccinimidyl(4-iodoacteyl)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-carbonyl)amino) hexanoate (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 (M_2C_2H), 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-(ρ-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-(ρ-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), ρ-nitrophenyl diazopyruvate (ρNPDP), ρ-nitrophenyl-2-diazo-3,3,3-trifluoropropionate (PNP-DTP), sulfhydryl-reactive and photoreactive cross-linkers such as1-(ρ-Azidosalicylamido)-4-(iodoacetamido)butane (AsIB), N-[4-(ρ-azidosalicylamido)butyl]-3′-(2′-pyridyldithio)propionamide (APDP), benzophenone-4-iodoacetamide, benzophenone-4-maleimide carbonyl-reactive and photoreactive cross-linkers such as ρ-azidobenzoyl hydrazide (ABH), carboxylate-reactive and photoreactive cross-linkers such as 4-(ρ-azidosalicylamido)butylamine (AsBA), and arginine-reactive and photoreactive cross-linkers such as ρ-azidophenyl glyoxal (APG).

[0341] 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.

[0342] In some embodiments, the linker comprises a maleimide goup. 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.

[0343] 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.

[0344] 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 aminoa cid 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: 2111), 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: 2112), or Gly-Phe-Leu-Gly (SEQ ID NO: 2113). In some instances, the linker comprises a peptide moiety such as: Val-Cit (valine-citrulline), Gly-Gly-Phe-Gly (SEQ ID NO: 2111), 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: 2112), or Gly-Phe-Leu-Gly (SEQ ID NO: 2113). In some cases, the linker comprises Val-Cit. In some cases, the linker is Val-Cit.

[0345] 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).

[0346] 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.

[0347] 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.

[0348] 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.

[0349] 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.

[0350] 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 heterobifuctional linker, such as a heterobifuctional linker described above. In some cases, a linker that exhibits steric hinderance comprises SMCC and SPDB.

[0351] 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).

[0352] 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.

[0353] 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.

[0354] 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 C_1-C_6 alkyl group. In some cases, X is a C_1-C_6 alkyl group, such as for example, a C_5, C_4, C_3, C_2, or C_1 alkyl group. In some cases, the C_1-C_6 alkyl group is an unsubstituted C_1-C_6 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 homobifuctional linker or a heterobifuctional linker described *supra*. In some cases, X includes a heterobifunctional linker. In some cases, X includes sMCC. In other instances, X includes a heterobifuctional linker optionally conjugated to a C_1-C_6 alkyl group. In other instances, X includes sMCC optionally conjugated to a C_1-C_6 alkyl group. In additional instances, X does not include a homobifuctional linker or a heterobifunctional linker described *supra*.

[0355] 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 C_1-C_6 alkyl group. In some instances, Y is a homobifuctional linker or a heterobifunctional linker described *supra*. In some instances, Y is a homobifuctional 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.

[0356] 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 C_1-C_6 alkyl group. In some instances, L is a homobifuctional linker or a heterobifunctional linker described *supra*. In some instances, L is a homobifuctional 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

[0357] 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 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

[0358] 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.

[0359] In some embodiments, the cancer is a solid tumor. Exemplary solid tumor includes, but is not limited to, 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.

[0360] In some instances, 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 solid tumor. In some instances, 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 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.

[0361] In some instances, the cancer is a hematologic malignancy. In some instances, the hematologic malignancy is 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.

[0362] In some instances, 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 hematologic malignancy. In some instances, 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 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.

[0363] 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 a binding moiety conjugated to a polynucleic acid molecule and a polymer 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 a binding moiety conjugated to a polynucleic acid molecule and a polymer is used for the treatment of a KRAS-associated cancer. In some instances, 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 an EGFR-associated cancer. In some instances, 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 an AR-associated cancer. In some instances, 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 an HPRT1-associated cancer. In some instances, 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 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

[0364] 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-idiotype, 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 elicite an immune response.

[0365] 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.

[0366] 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.

[0367] 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.

[0368] 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.

[0369] 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.

[0370] In some embodiments, exemplary checkpoint inhibitors include:

[0371] 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;

[0372] PD-L2 inhibitors such as GlaxoSmithKline's AMP-224 (Amplimmune), and rHIgM12B7;

[0373] 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;

[0374] 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;

[0375] LAG3 inhibitors such as anti-Lag-3 antibody clone eBioC9B7W (C9B7W) from eBioscience, anti-Lag3 antibody LS-B2237 from LifeSpan Biosciences, IMP321 (ImmuFact) from Immutep, anti-Lag3 antibody BMS-986016, and the LAG-3 chimeric antibody A9H12;

[0376] B7-H3 inhibitors such as MGA271;

[0377] KIR inhibitors such as Lirilumab (IPH2101);

[0378] CD137 (41BB) inhibitors such as urelumab (BMS-663513, Bristol-Myers Squibb), PF-05082566 (anti-4-1BB, PF-2566, Pfizer), or XmAb-5592 (Xencor);

[0379] PS inhibitors such as Bavituximab;

[0380] 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.

[0381] 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 collageneous 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).

[0382] 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).

[0383] 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.

[0384] 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.

[0385] 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.

[0386] 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, TNFα, 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).

[0387] 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-1β, IL-2, IL-7, IL-8, IL-15, MCP-1 (CCL2), MIP-1α, RANTES, MCP-3, MIP5, CCL19, CCL21, CXCL2, CXCL9, CXCL10, or CXCL11 is used for the treatment of a disease or disorder (e.g., cancer).

[0388] 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-idiotype 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).

[0389] 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.

[0390] 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).

[0391] 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).

[0392] 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).

[0393] 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.

[0394] 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).

Pharmaceutical Formulation

[0395] 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 describe herein is formulated for parenteral (e.g., intravenous, subcutaneous, intramuscular) administration. In other instances, the pharmaceutical composition describe herein is formulated for oral administration. In still other instances, the pharmaceutical composition describe herein is formulated for intranasal administration.

[0396] 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.

[0397] 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.

[0398] In some instances, a nanoparticle includes a core or a core and a shell, as in a core-shell nanoparticle.

[0399] 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, carragheenan, fucoidan, agaropectin, porphyran, 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.

[0400] In some cases, a nanoparticle has at least one dimension of less than about 500nm, 400nm, 300nm, 200nm, or 100nm.

[0401] 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.

[0402] 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, polyvinylpyrrollidone (PVP), cholesterol, cholesterol esters, sodium caseinate, soy lecithin, taurocholic acid, phosphotidylcholine, sodium chloride, tricalcium phosphate, dipotassium phosphate, cellulose and cellulose conjugates, sugars sodium stearoyl 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 Fo*rms *and* Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins1999).

[0403] 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.

[0404] 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.

[0405] 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.

[0406] 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.

[0407] 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.

[0408] 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.

[0409] 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.

[0410] Solubilizers include compounds such as triacetin, triethylcitrate, ethyl oleate, ethyl caprylate, sodium lauryl sulfate, sodium doccusate, 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, transcutol, propylene glycol, dimethyl isosorbide, and the like.

[0411] Stabilizers include compounds such as any antioxidation agents, buffers, acids, preservatives and the like.

[0412] 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.

[0413] 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.

[0414] 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.

[0415] 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

[0416] 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.

[0417] In some embodiments, one or more pharmaceutical compositions are administered simutaneously, sequentially, or at an interval period of time. In some embodiments, one or more pharmaceutical compositions are administered simutaneously. 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).

[0418] In some embodiments, two or more different pharmaceutical compositions are coadministered. In some instances, the two or more different pharmaceutical compositions are coadministered simutaneously. 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.

[0419] 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%.

[0420] 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.

[0421] 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.

[0422] 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.

[0423] 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

[0424] 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.

[0425] 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.

[0426] For example, the container(s) include a molecule of Formula (I): A-X-B-Y-C, optionally conjugated to an endosomolytic moiety D as disclosed herein. Such kits optionally include an identifying description or label or instructions relating to its use in the methods described herein.

[0427] 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.

[0428] 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.

[0429] 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

[0430] 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.

[0431] 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 μL" means "about 5 μL" and also "5 μL." Generally, the term "about" includes an amount that is expected to be within experimental error.

[0432] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

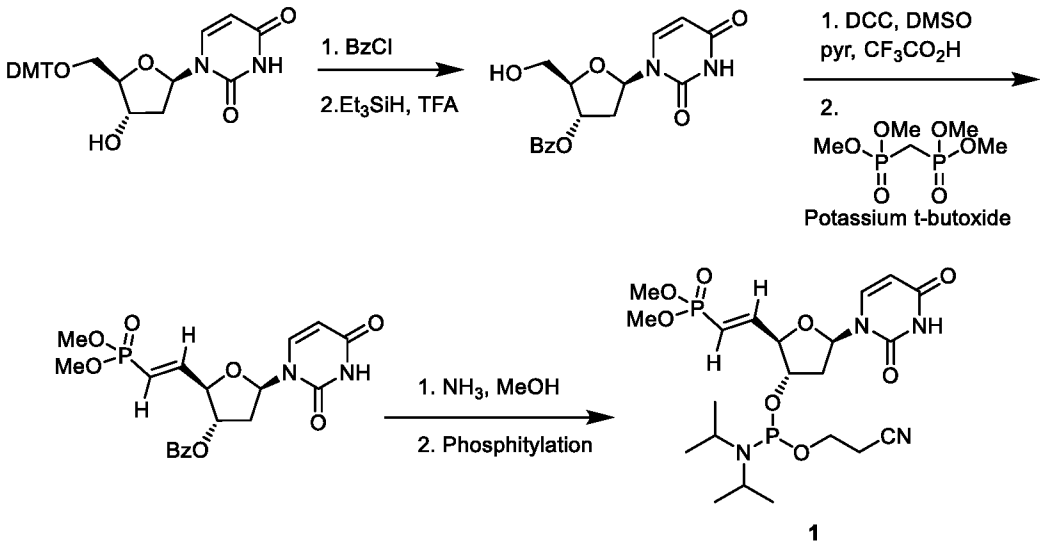
[0433] As used herein, the terms "individual(s)", "subject(s)" and "patient(s)" mean any mammal. In some embodiments, the mammal is a human. In some embodiments, the mammal is a non-human. None of the terms require or are limited to situations characterized by the supervision (e.g. constant or intermittent) 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).

EXAMPLES

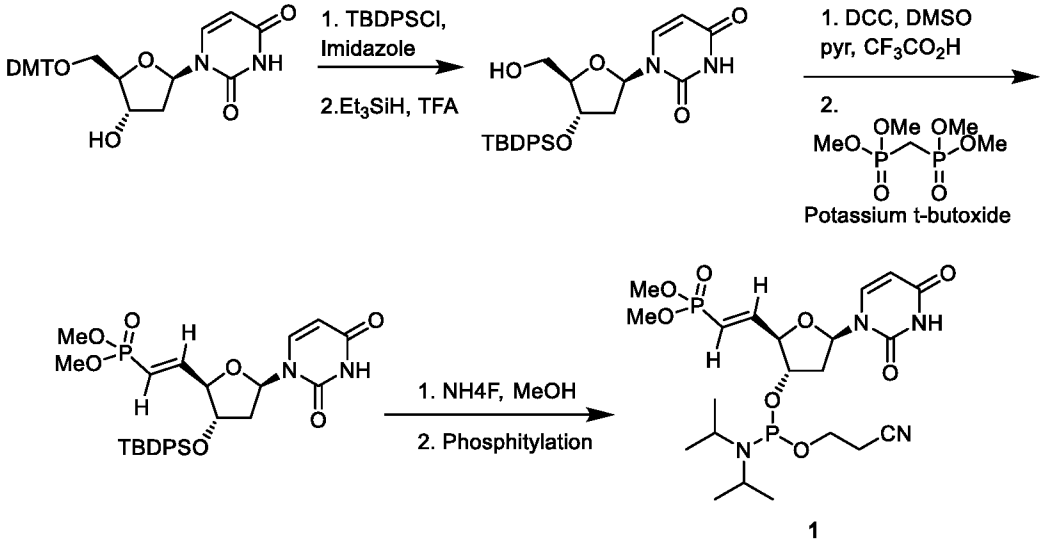
[0434] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

Chemical Synthesis Examples

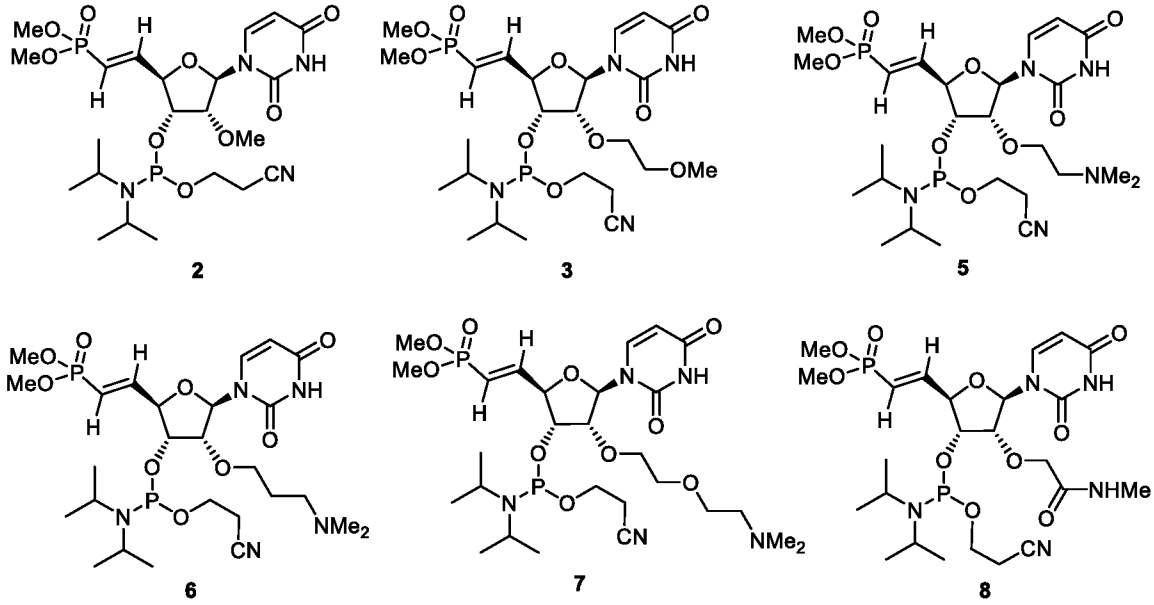
[0435] Example 1. Preparation of Compound 1-3, and 5-8



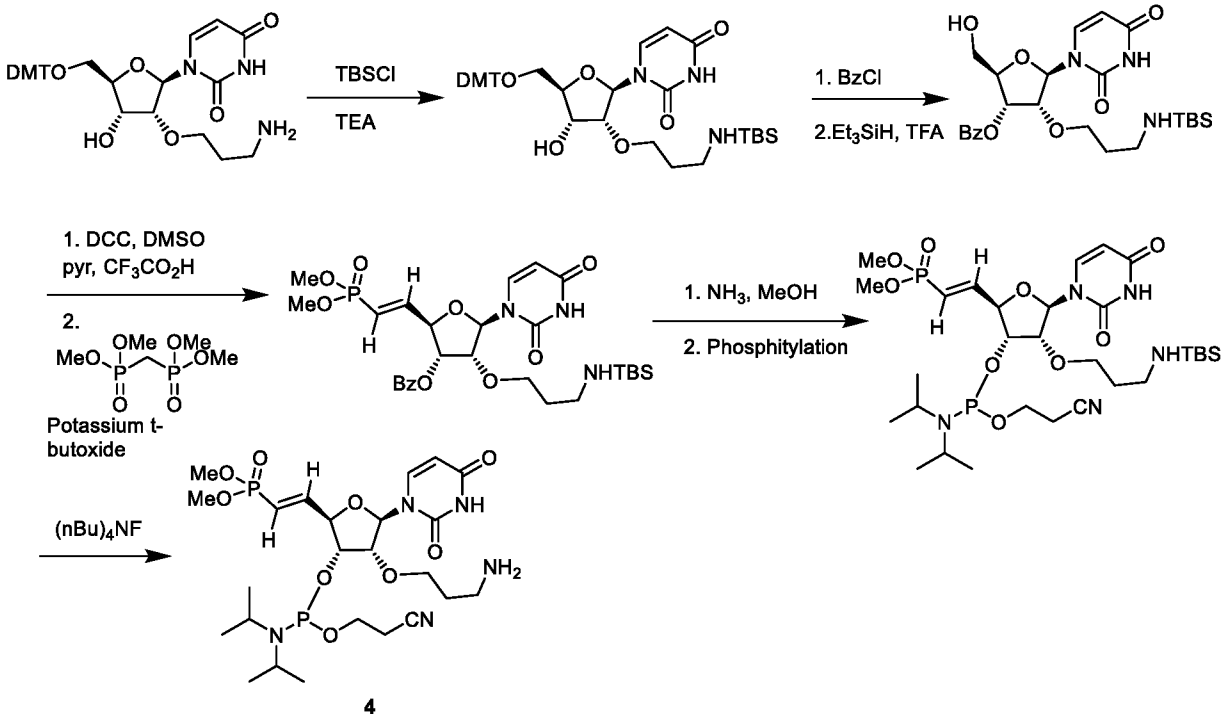
or as follows



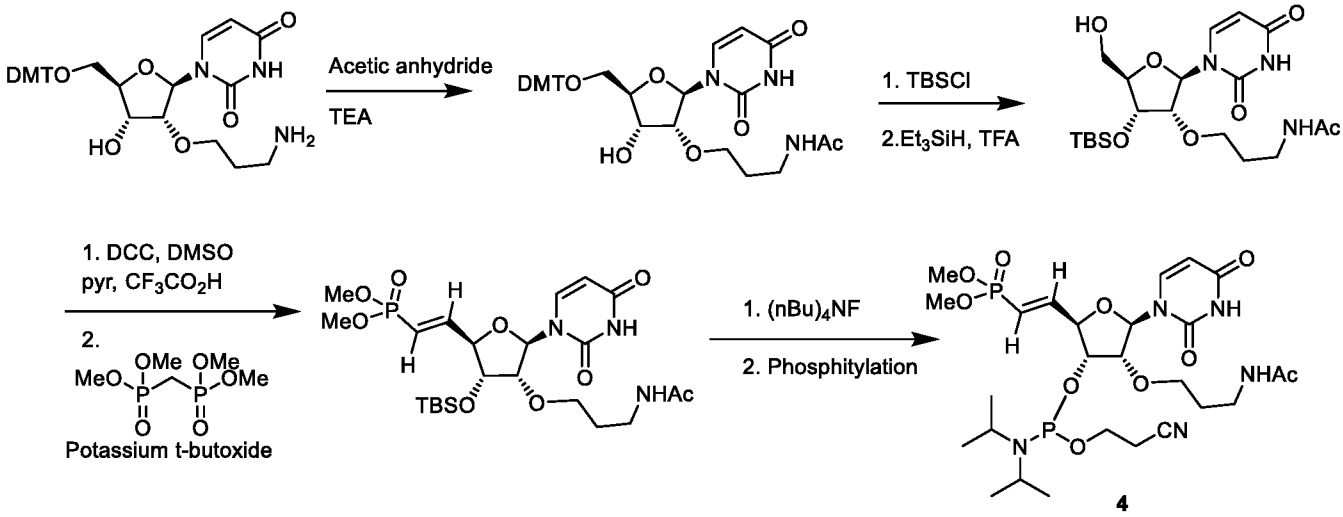
Compounds 2, 3, and 5-8 were prepared as per procedures illustrated in Example 1.



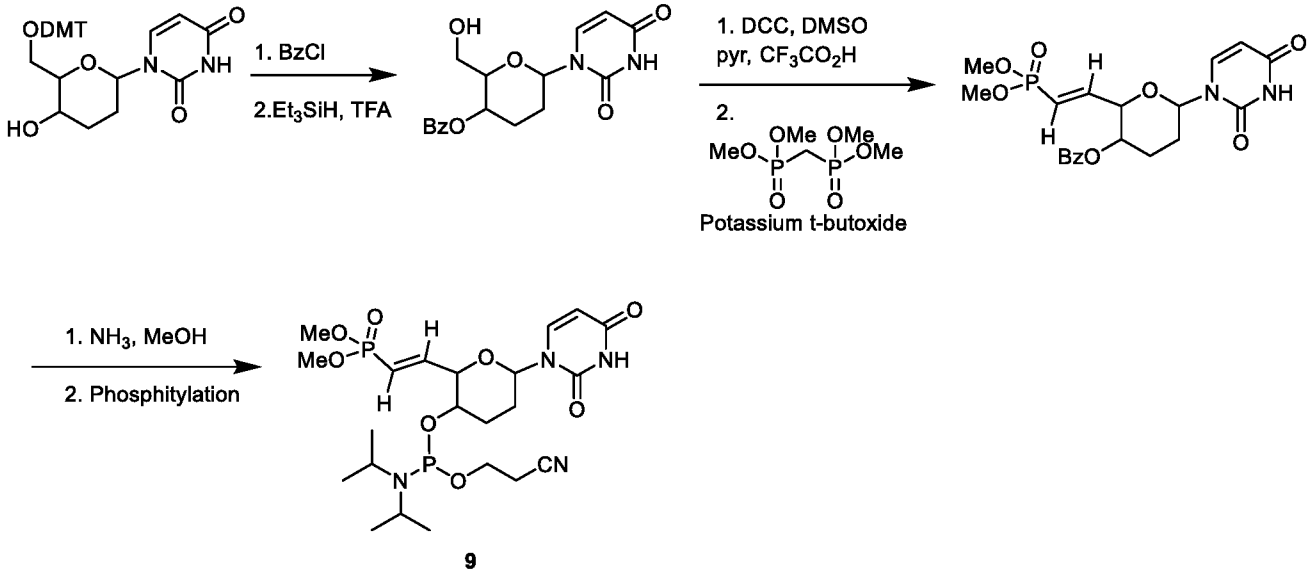
[0436] Example 2. Preparation of Compound 4



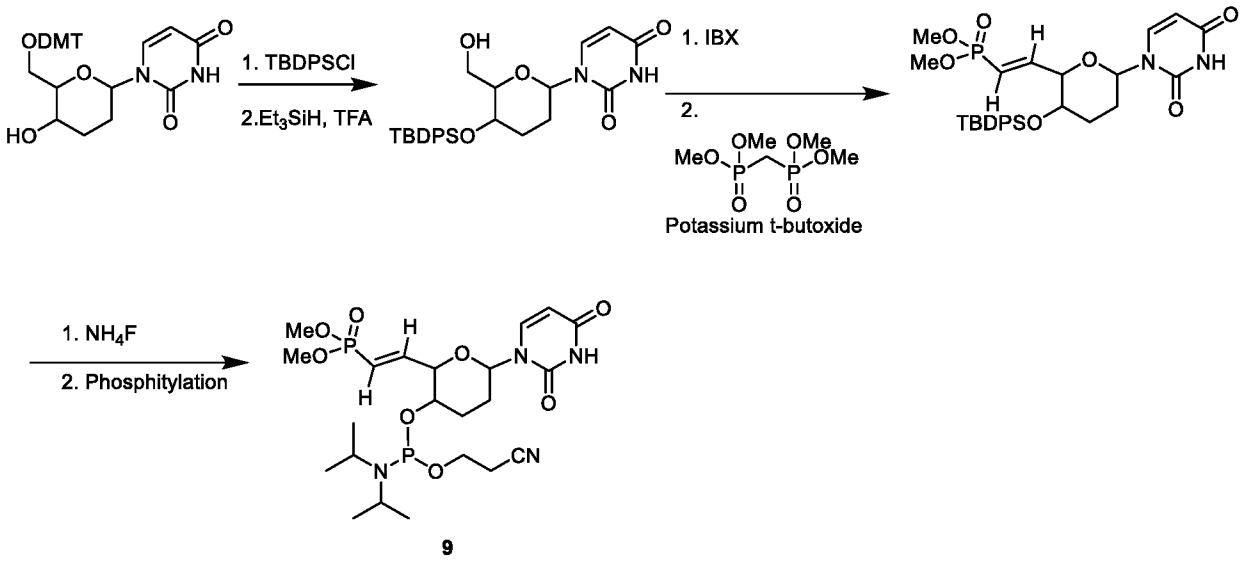
or as follows:



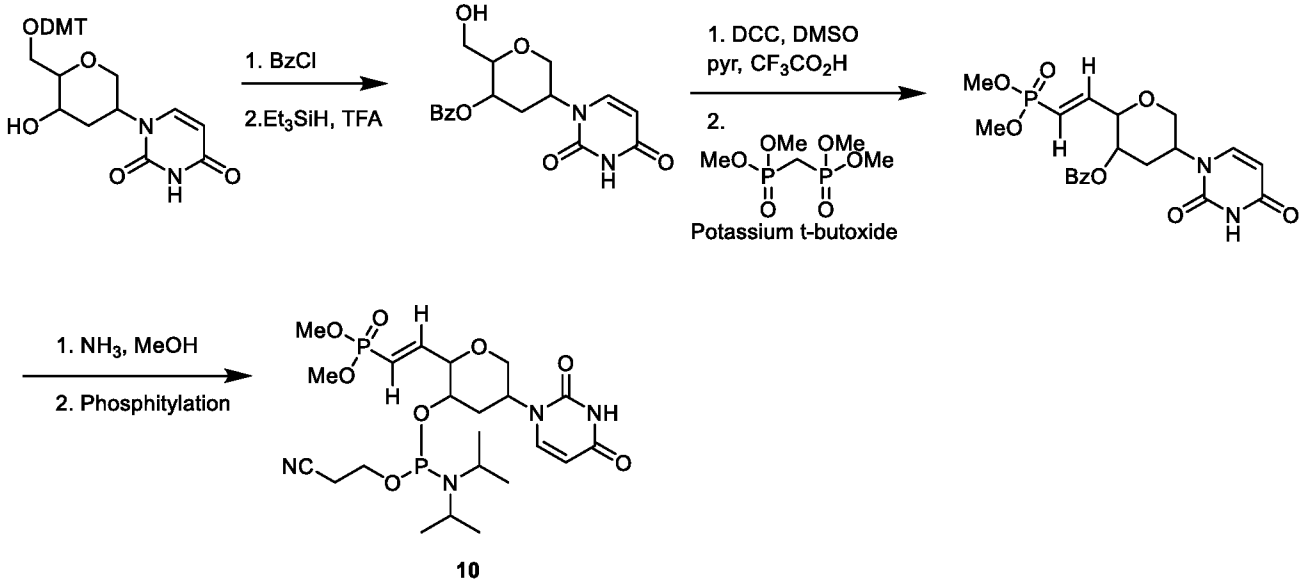
[0437] Example 3. Preparation of Compound 9



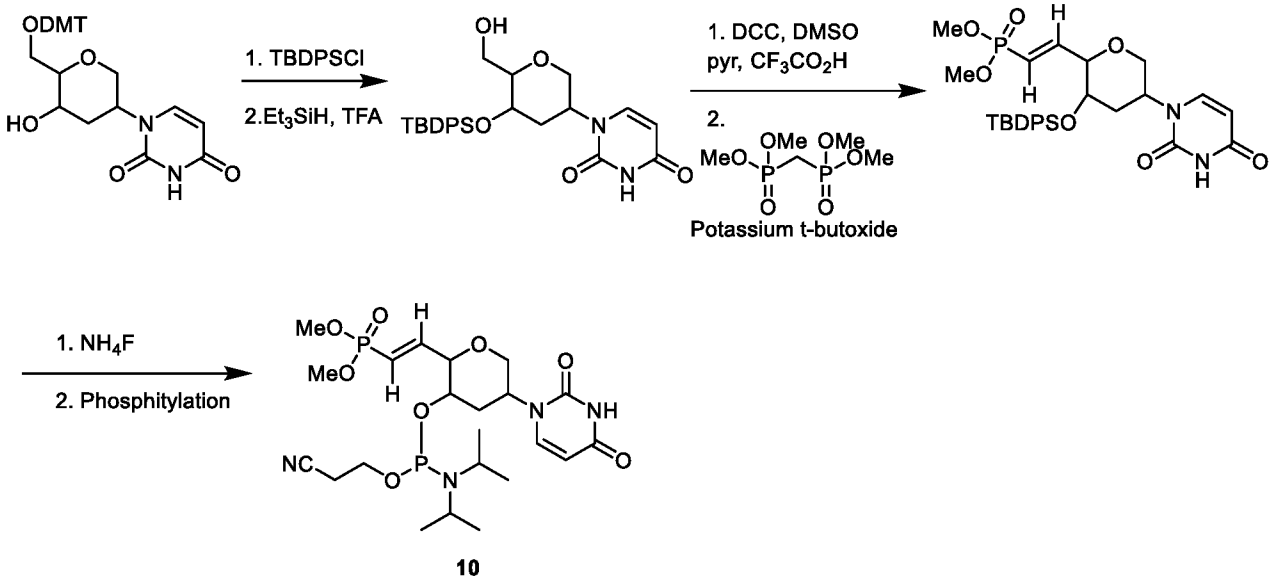
or as follows:



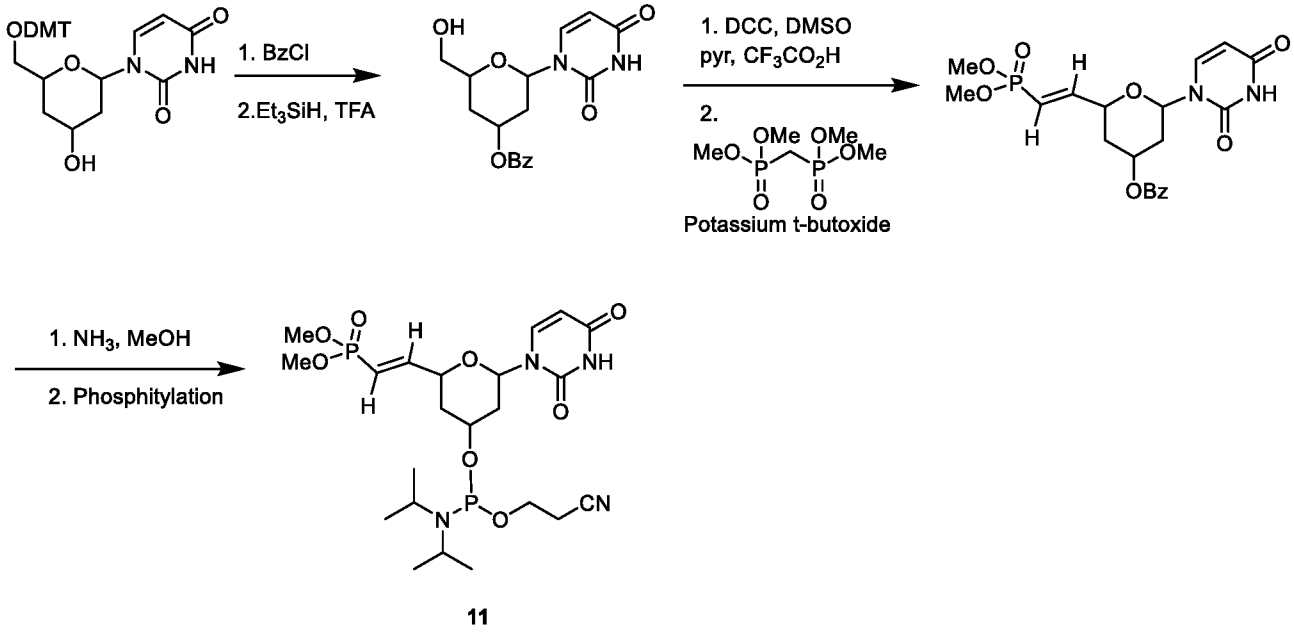
[0438] Example 4. Preparation of Compound 10



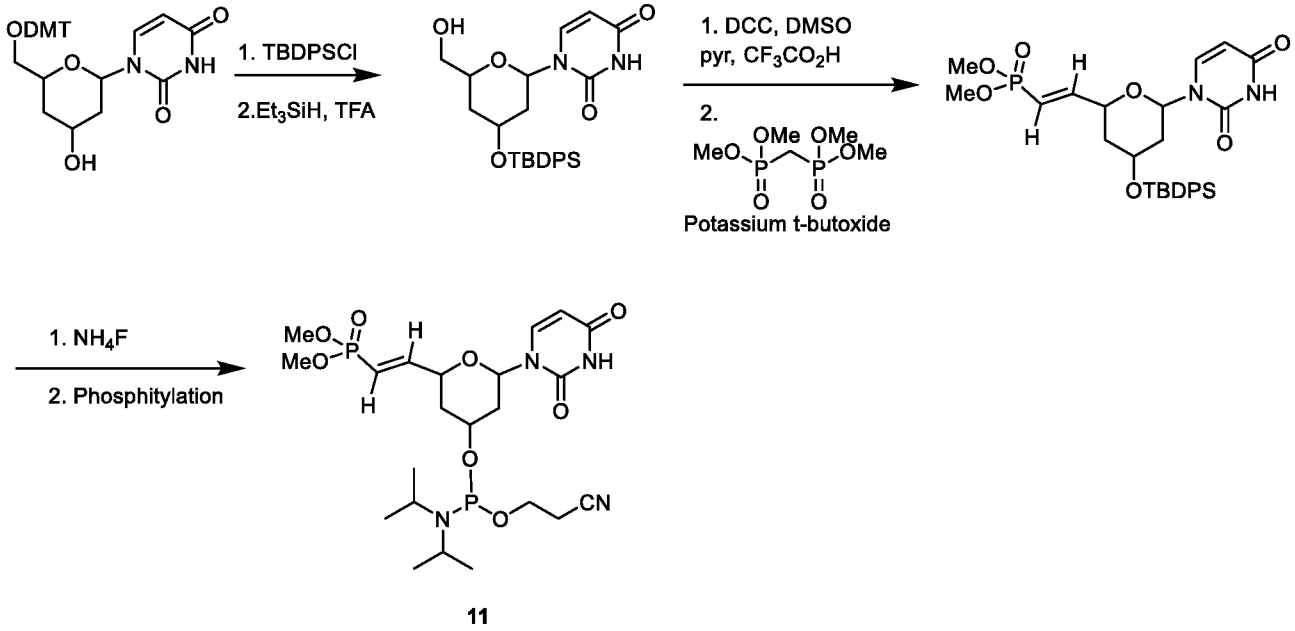
or as follows:



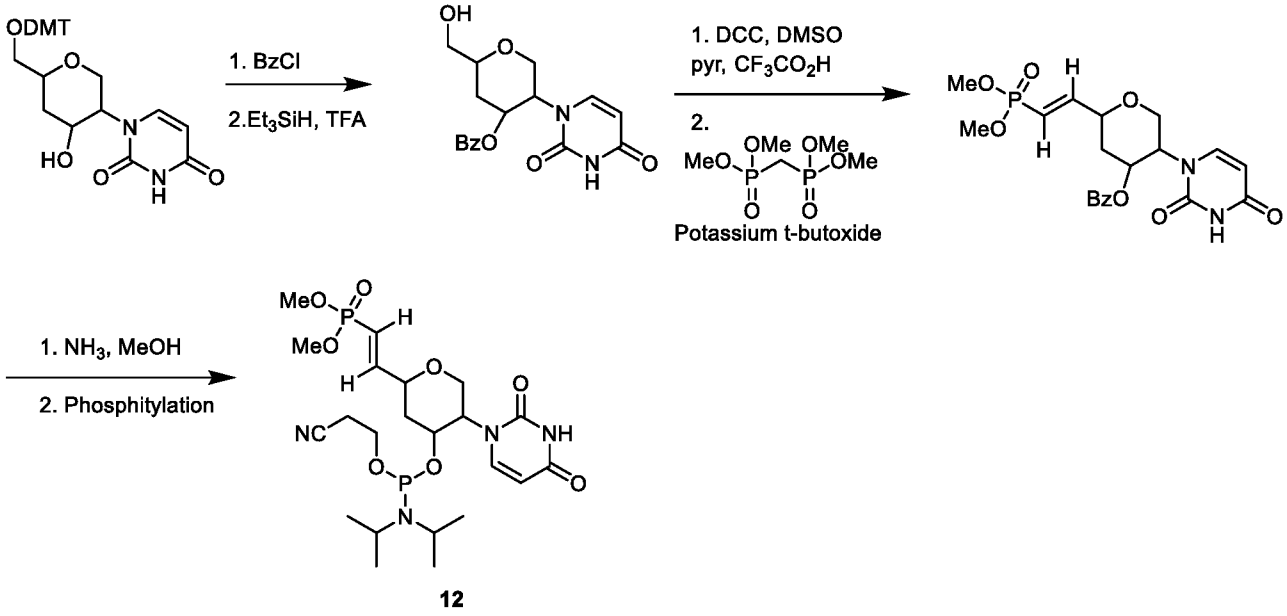
[0439] Example 5. Preparation of Compound 11



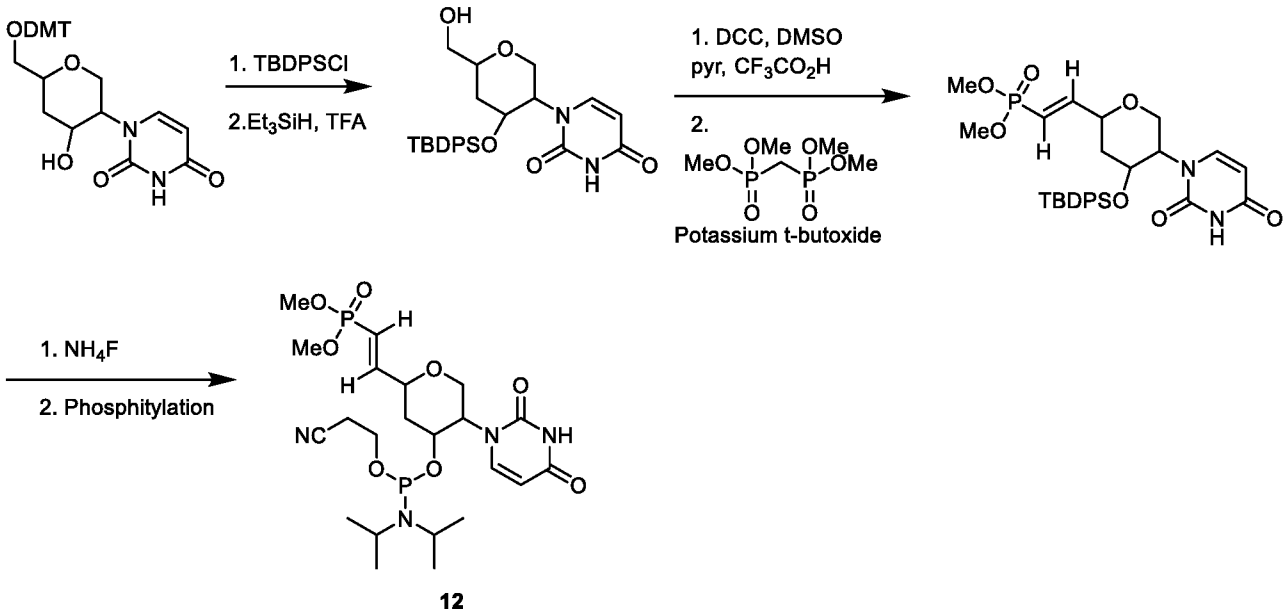
or as follows:



[0440] Example 6. Preparation of Compound 12

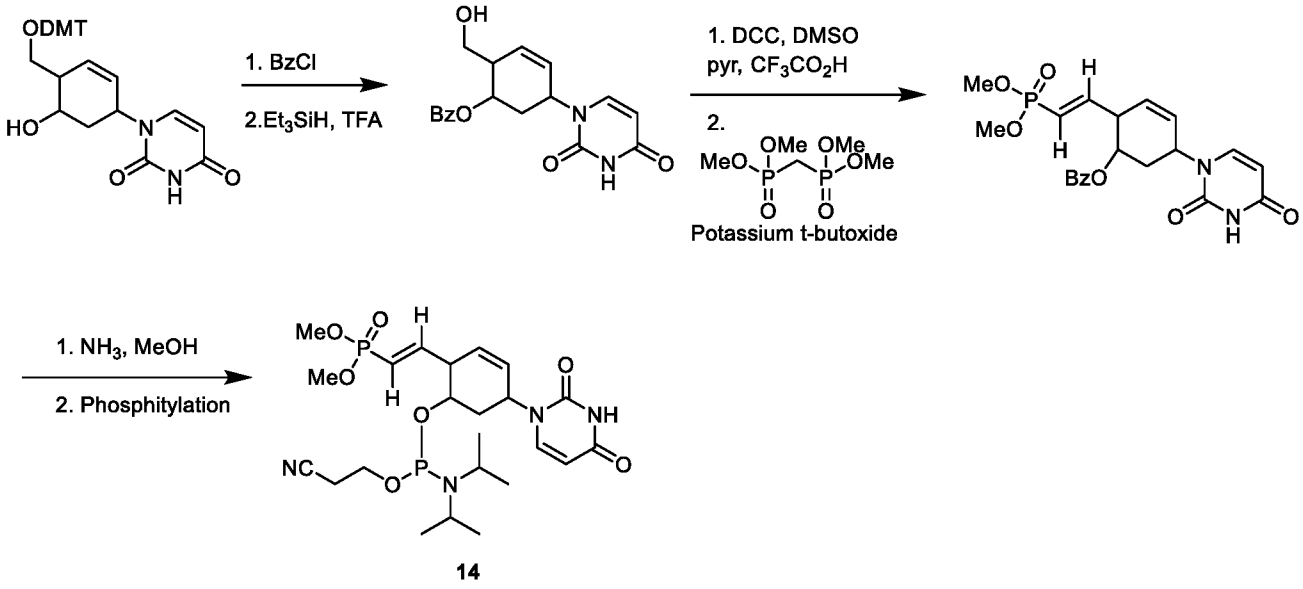


or as follows:



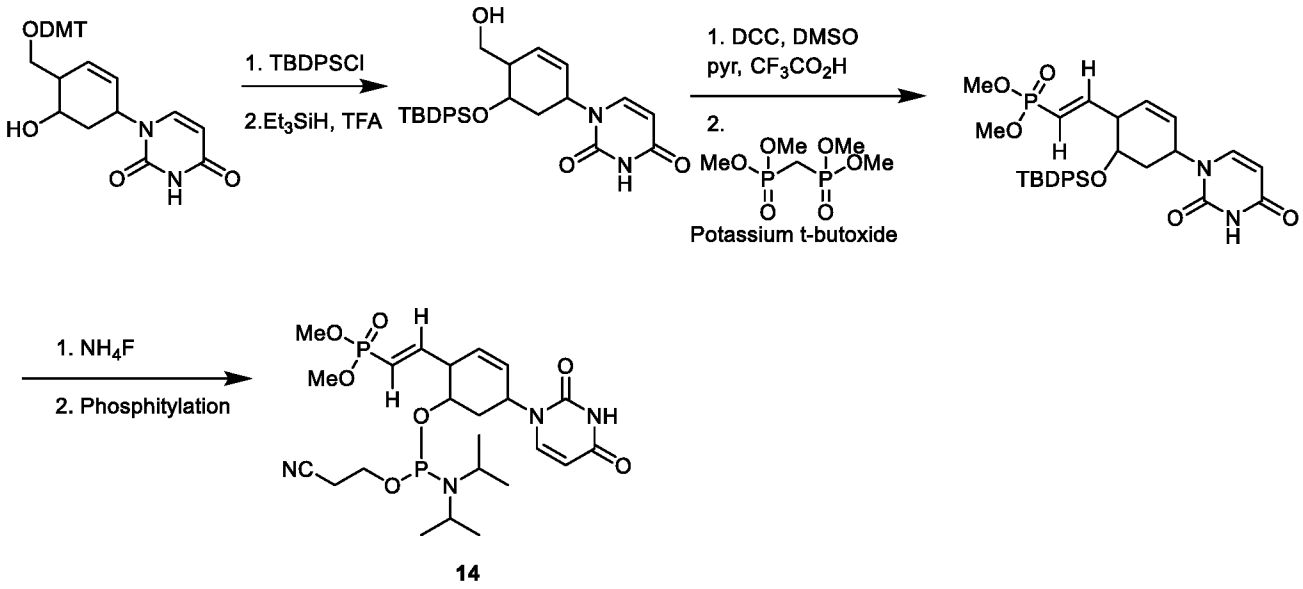
12

[0441] Example 7. Preparation of Compound 14

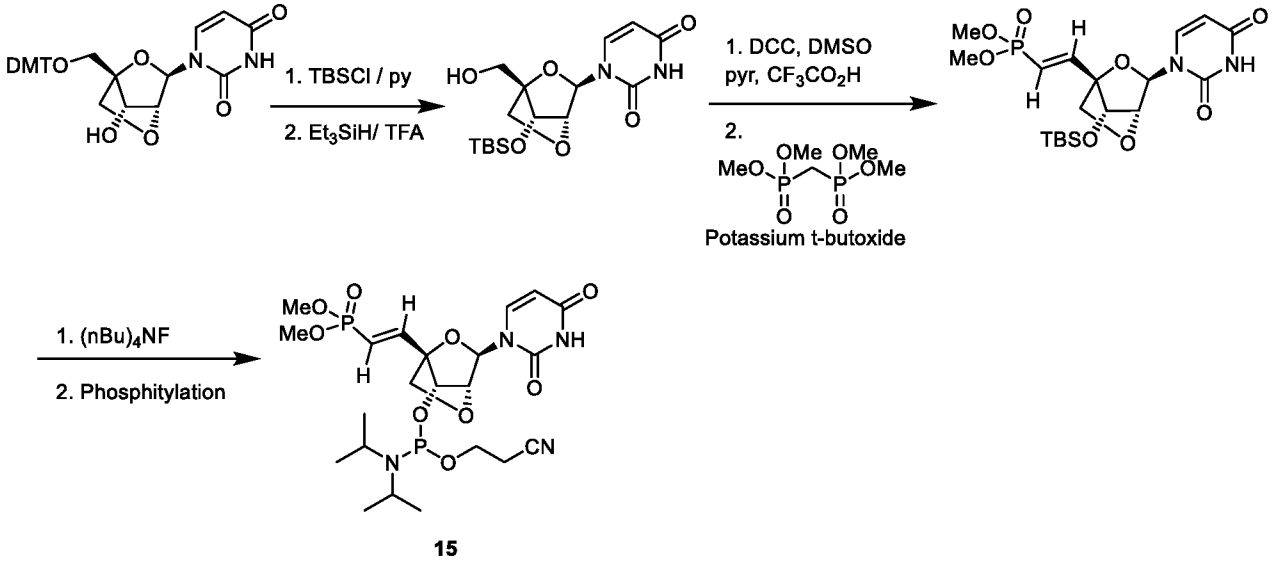


14

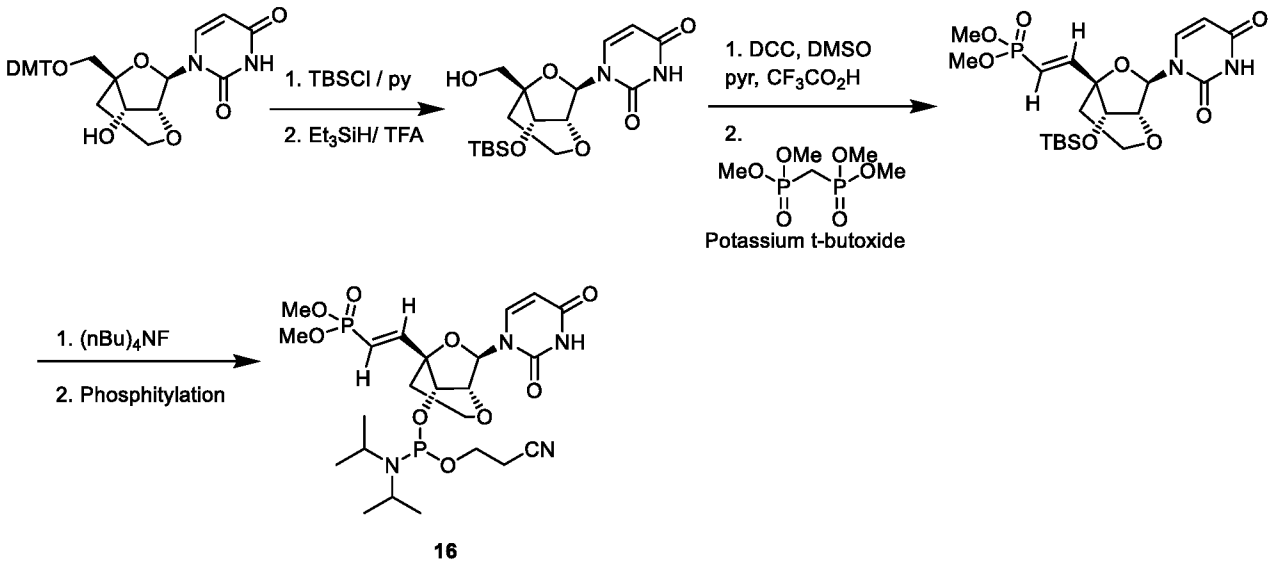
or as follows:



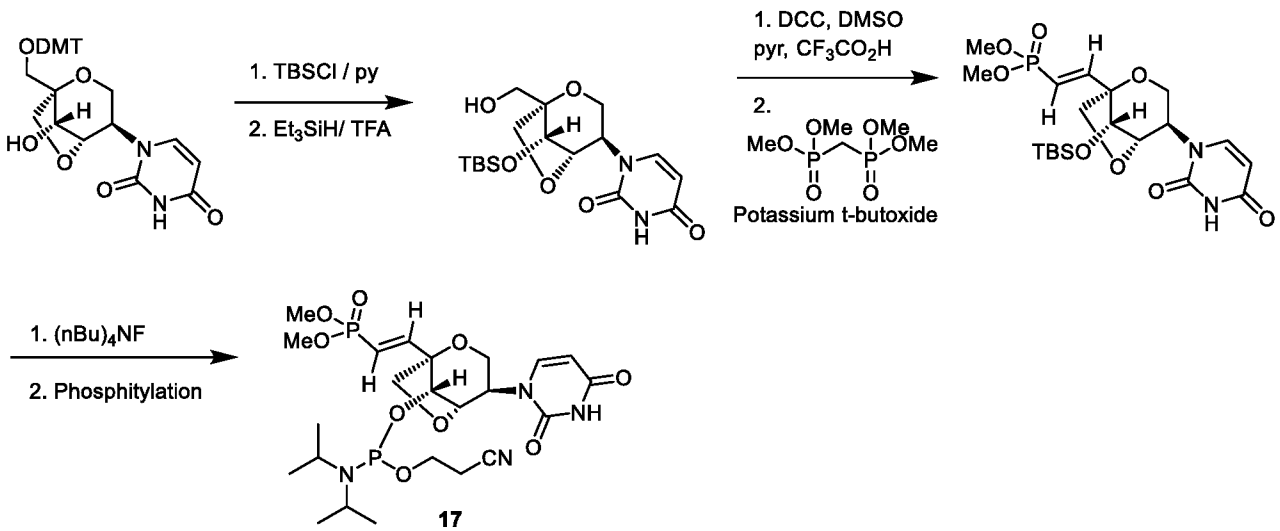
[0442] Example 8. Preparation of Compound 15



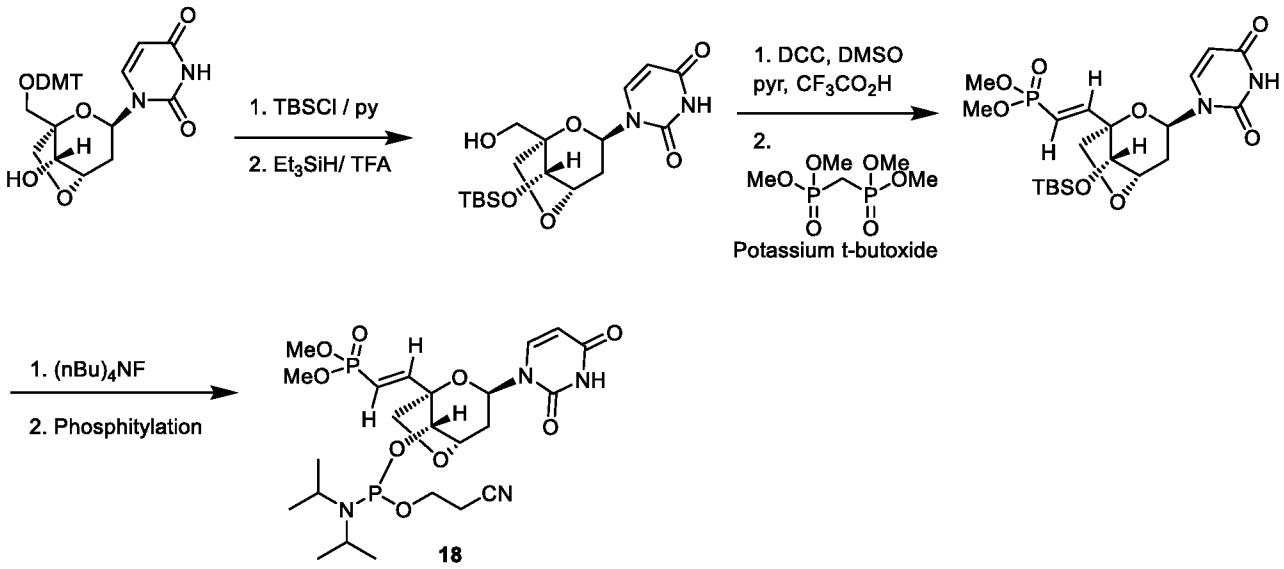
[0443] Example 9. Preparation of Compound 16



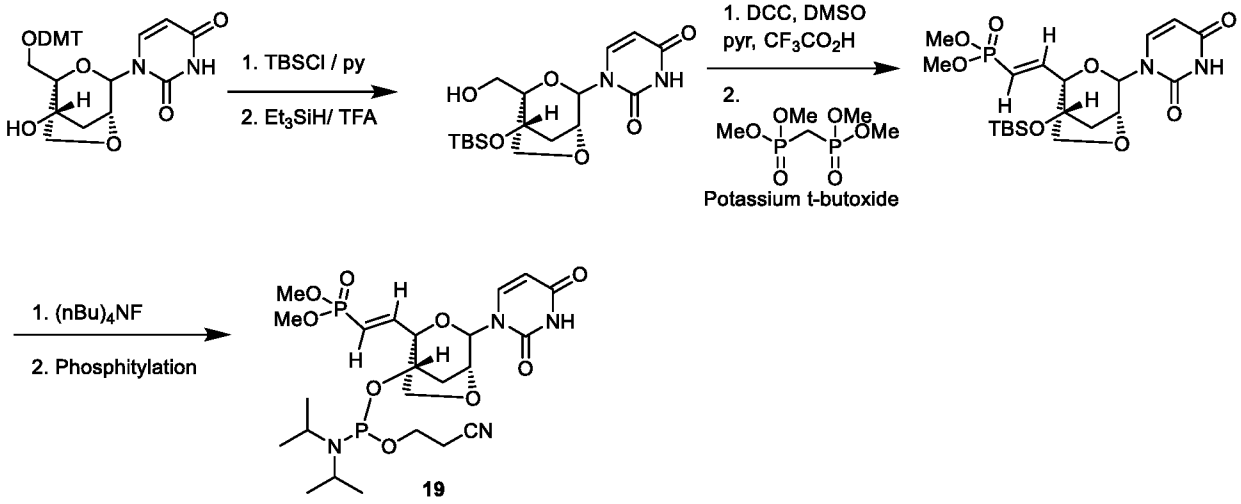
[0444] Example 10. Preparation of Compound 17



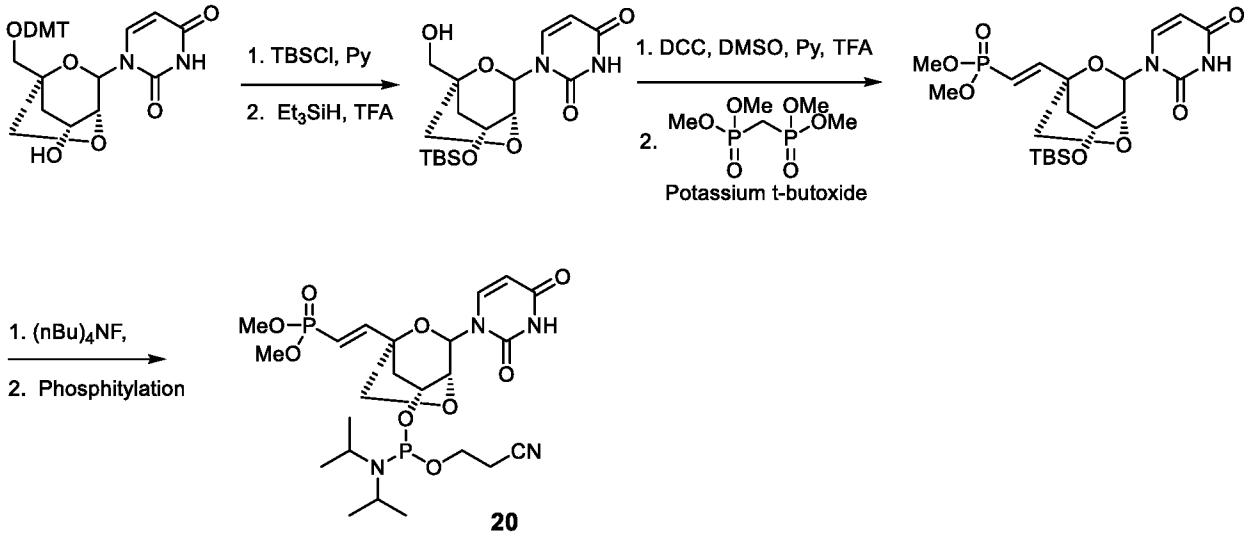
[0445] Example 11. Preparation of Compound 18



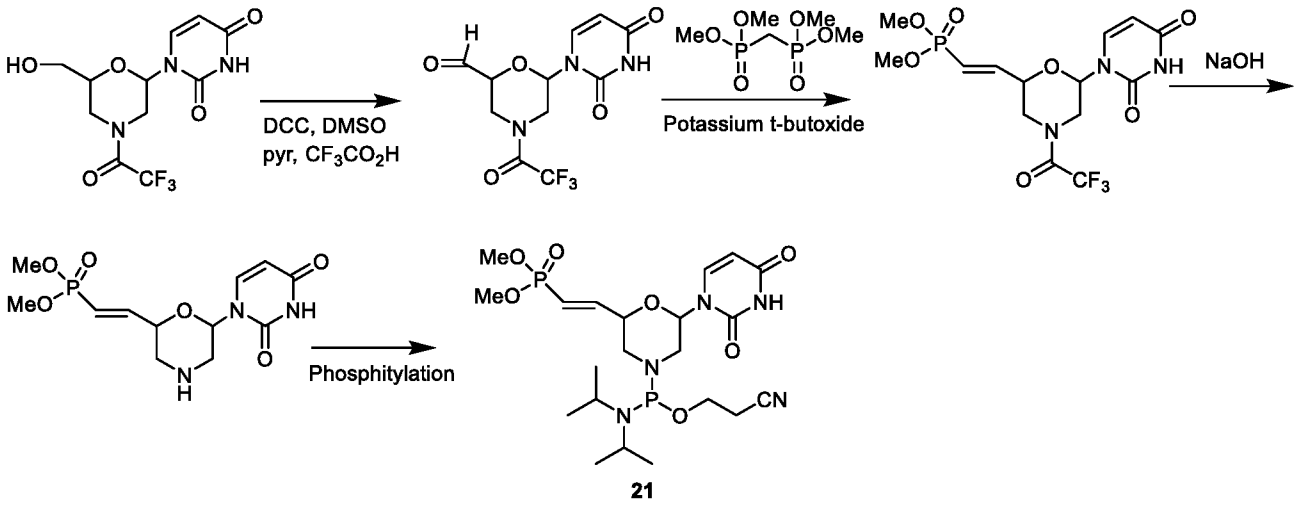
[0446] Example 12. Preparation of Compound 19



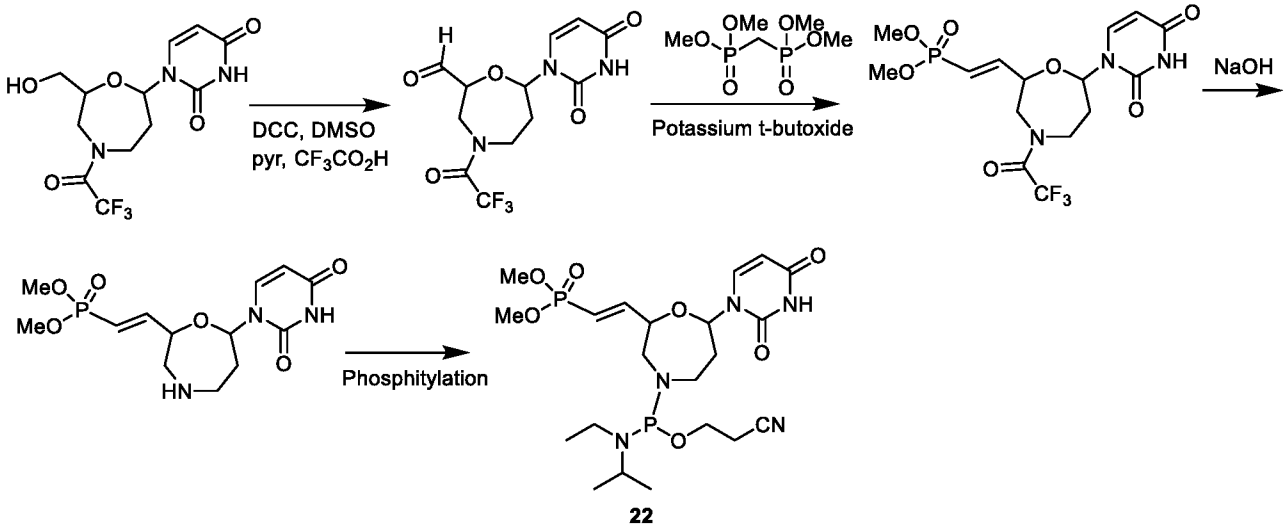
[0447] Example 13. Preparation of Compound 20



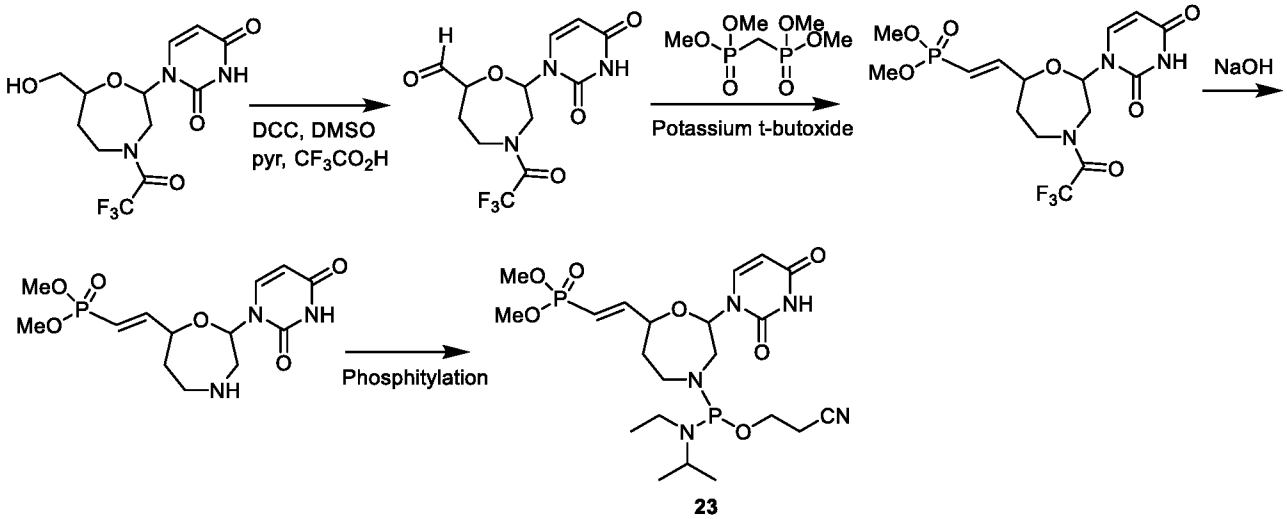
[0448] Example 14. Preparation of Compound 21



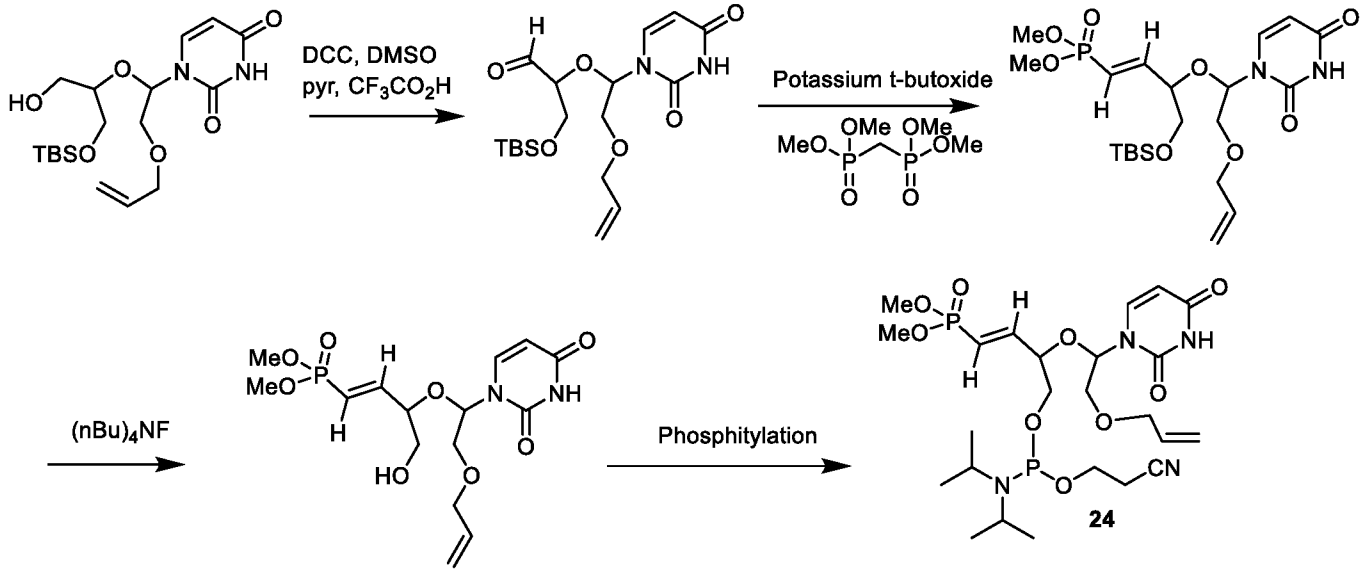
[0449] Example 15. Preparation of Compound 22



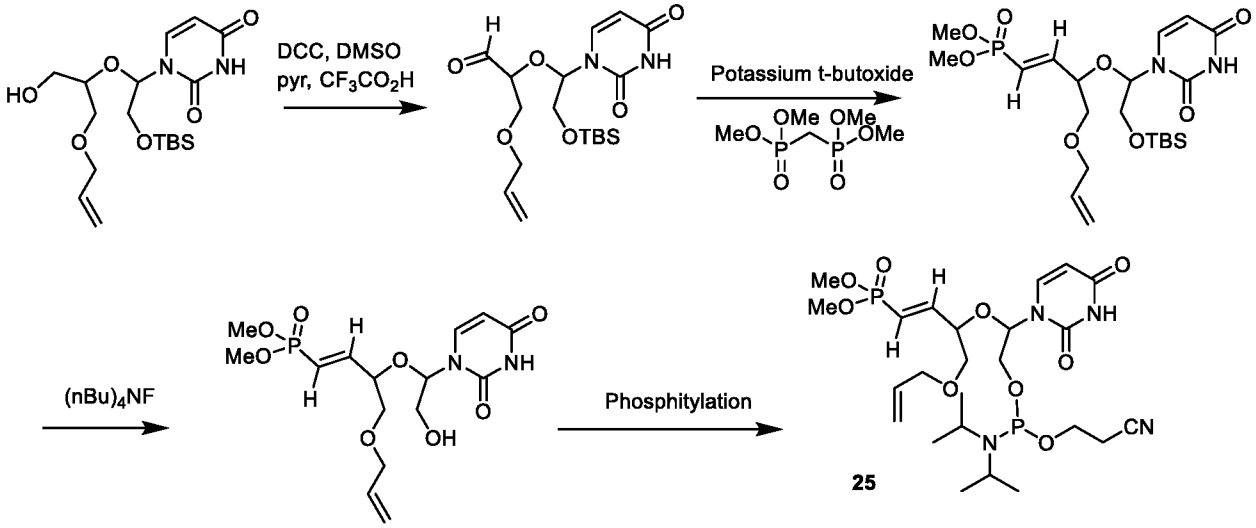
[0450] Example 16. Preparation of Compound 23



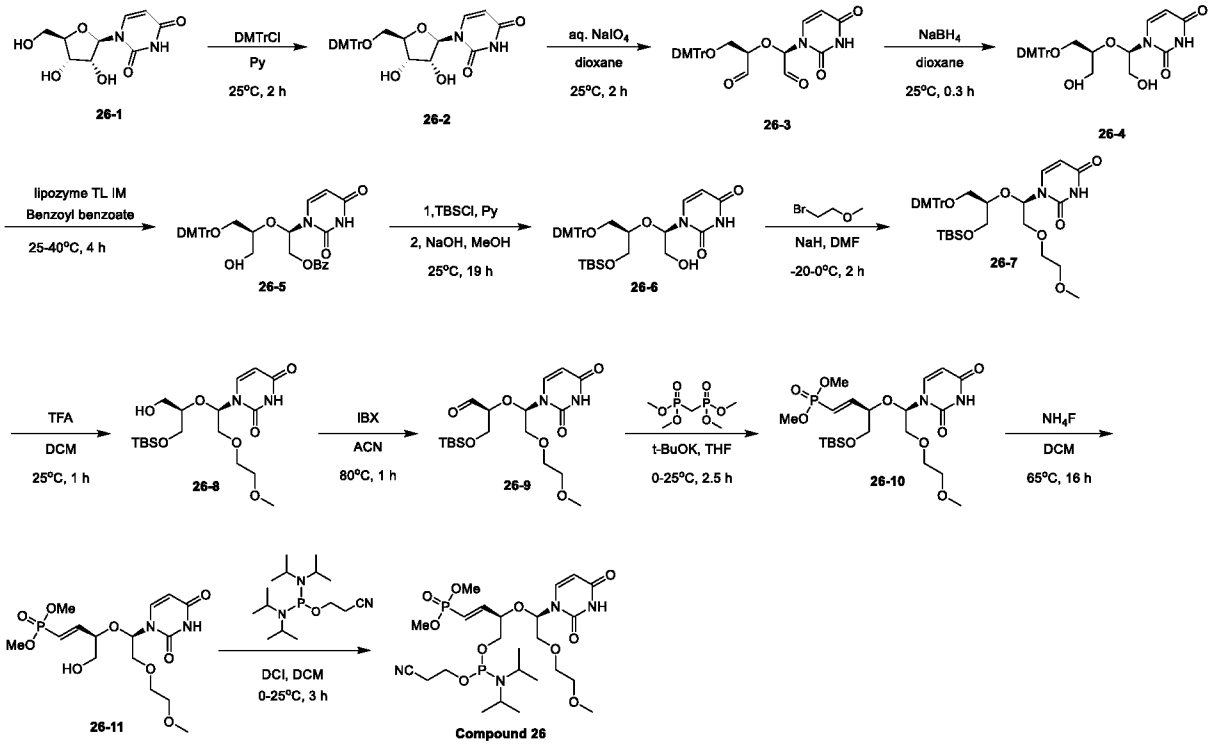
[0451] Example 17. Preparation of Compound 24



[0452] Example 18. Preparation of Compound 25

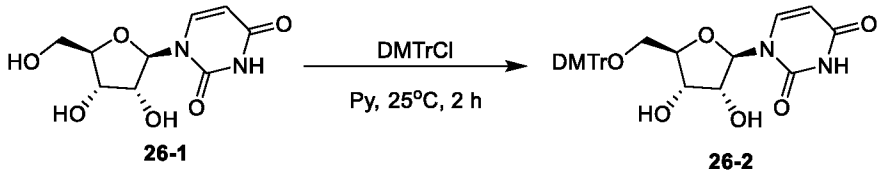


[0453] Example 19. Preparation of Compound 26



[0454] Synthetic procedures for the preparation of compound 26

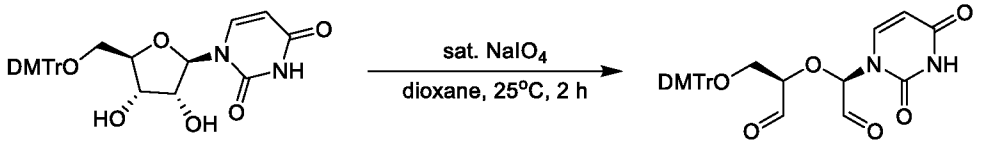
[0455] Compound 26-2



To a solution of compound **26-1** (500 g, 2.05 mol, 1.00 eq) in Py (3500 mL) was added DMTrCl (763 g, 2.25 mol, 1.10 eq). The mixture was stirred at 25°C for 2 h. TLC (DCM/MeOH = 10/1, compound **26-2**: R_f = 0.60) indicated compound **26-1** was consumed completely. The reaction mixture was diluted with DCM (5.00L) and washed with $NaHCO_3$ (2.00 L x 2). The combined organic layers were washed with Brine (2.00 L), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate/TEA = 50/1/0.5% to 0/1/0.5%). Compound **26-2** (700 g, 56.3% yield, 90.0% purity) was obtained as a white solid.

^{1}H NMR: 400 MHz, DMSO-d_6 δ ppm 11.35 (s, 1 H), 7.72 (d, J = 8.0 Hz, 1H), 7.36-7.41 (m, 2H), 7.32 (t, J = 7.6 Hz, 2H), 7.20-7.28 (m, 5H), 6.90 (d, J = 8.6 Hz, 4H), 5.48 (d, J = 4.8 Hz, 1H), 5.31 (d, J = 8.0 Hz, 1H), 5.14 (d, J = 5.6 Hz, 1H), 4.09 (q, J = 5.4 Hz, 2H), 3.92-3.99 (m, 1H), 3.70-3.78 (m, 6H), 3.18-3.30 (m, 2H).

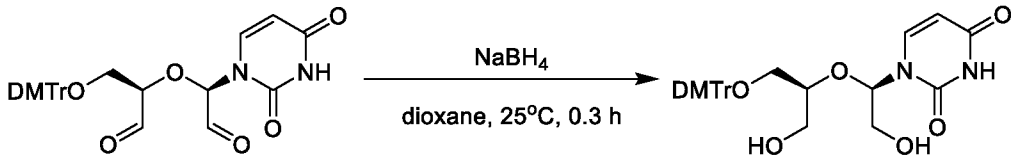
[0456] Compound 26-3



26-2 26-3

To a solution of compound **26-2** (250 g, 457 mmol, 1.00 eq) in dioxane (2500 mL) was added sat $NaIO_4$ (103 g, 484 mmol, 4.85 mL, 1.06 eq) solution. The mixture was stirred at 25°C for 2 h. TLC (DCM/MeOH = 10/1, compound **26-3**: R_f = 0.49) indicated compound **26-2** was consumed completely. The reaction mixture was filtered. The crude product compound **26-3** (250 g) was obtained as a colorless oil and used in the next step without further purification.

[0457] Compound 26-4

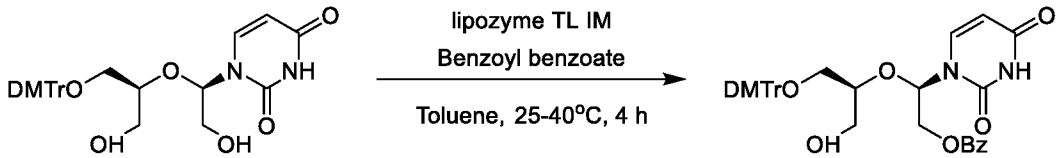


26-3 26-4

To a solution of compound **26-3** (250 g, 459 mmol, 1.00 eq) in dioxane (3000 mL) was added $NaBH_4$ (17.3 g, 459 mmol, 1.00 eq). The mixture was stirred at 25°C for 0.3 h. TLC (DCM/MeOH = 10/1, compound **26-4**: R_f = 0.41) indicated compound **26-3** was consumed completely. The reaction mixture was quenched with acetone, neutralized with 20% acetic acid, and concentrated to give a residue under reduced pressure. The residue was diluted with DCM (2.00 L), washed with H_2O (2.00 L), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The product was used into the next step without further purification. Compound **26-4** (250 g) was obtained as a colorless oil.

^{1}H NMR: 400 MHz, DMSO-d_6 δ ppm 11.35 (s, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.28-7.33 (m, 4H), 7.17-7.21 (m, 5H), 6.86 (d, J = 8.0 Hz, 4H), 5.83 (t, J = 6.4 Hz, 1H), 5.53 (d, J = 8.0 Hz, 1H), 5.13 (t, J = 8.0 Hz, 1H), 4.75 (t, J = 5.2 Hz, 1H), 3.76 (s, 6H), 3.68-3.73 (m, 1H), 3.60-3.64 (m, 2H), 3.42 (t, J = 5.2 Hz, 2H), 2.96-3.02 (m, 2H)

[0458] Compound 26-5

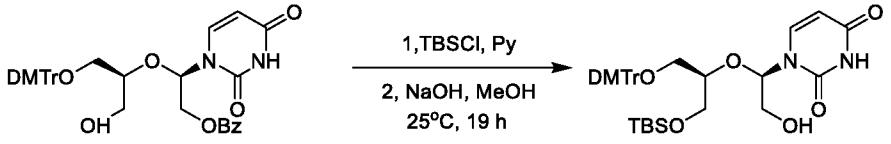


26-4 26-5

To a solution of compound **26-4** (50.0 g, 91.1 mmol, 1.00 eq) in toluene (2500 mL) was added benzoyl benzoate (30.9 g, 136 mmol, 25.7 mL, 1.50 eq) and lipozyme TL IM (30.0 g, 29.2

mmol) at 25°C. The mixture was stirred at 40°C for 4 h. TLC (DCM/MeOH = 20/1, compound **26-5**: R_f = 0.58) indicated compound **26-4** was consumed completely. The reaction mixture was filtered, quenched by addition methanol (250 mL), concentrated under reduced pressure to give a residue, which was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate/TEA=10/1/0.5% to 1/2/0.5%). Compound **26-5** (37.5 g, 63.0% yield) was obtained as a white solid.

[0459] Compound 26-6

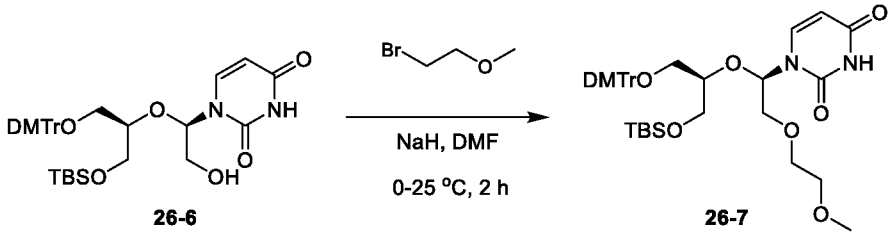


26-5 **26-6**

To a solution of compound **26-5** (340 g, 520 mmol, 1.00 eq) in Py (1700 mL) was added TBSCl (157 g, 1.04 mol, 127 mL, 2.00 eq) below 25°C. The mixture was stirred at 25°C for 19 h. TLC (DCM/MeOH = 20/1, compound **26-6**: R_f = 0.37) indicated compound **26-5** was consumed completely. Water (340 mL) was added to the reaction mixture. The resulting mixture containing the nucleoside was dissolved in MeOH (1.00 L), then added dropwise NaOH in MeOH (1.00 L, pH = 10) at 0°C, then stirred for 2.5 h at 0°C. The mixture was concentrated under reduced pressure to give a residue. Sat. aq NH_4Cl (1.00 L) was added to the mixture, which was stirred for 10 min. Water (1.00 L) was added and the mixture extracted with DCM (1.00 L), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate/TEA = 10/1/0.5% to 0/1/0.5%). Compound **26-6** (250 g, 339 mmol, 65.2% yield, 90.0% purity) was obtained as a white solid.

^{1}H NMR: 400 MHz, DMSO-d_6 δ ppm 11.35 (s, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.24-7.33 (m, 4H), 7.14-7.22 (m, 5H), 6.82-6.88 (m, 4H), 5.83 (t, J = 5.8 Hz, 1H), 5.47-5.57 (m, 1H), 5.11 (t, J = 5.8 Hz, 1H), 3.72 (d, J = 0.8 Hz, 6H), 3.57-3.67 (m, 4H), 3.46-3.55 (m, 1H), 2.94-3.00 (m, 2H), 0.72-0.78 (m, 9H), -0.04 (d, J = 8.2 Hz, 5H)

[0460] Compound 26-7

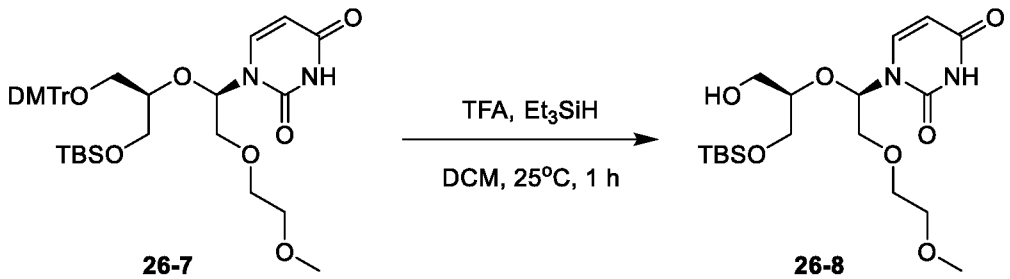


26-6 **26-7**

To a solution of compound **26-6** (50.0 g, 75.4 mmol, 1.00 eq) in DMF (500 mL) was added NaH (9.96 g, 248. mmol, 1.81 uL, 60% purity, 3.30 eq) at -20°C and stirred at -20°C for 0.5 h. Then the alkoxy bromide (15.7 g, 113. mmol, 10.6 mL, 1.50 eq) was added to the reaction. The mixture was stirred at 0°C for 1.5 h. TLC (Petroleum ether/Ethyl acetate = 1/2, compound 7: R_f = 0.43) indicated compound **26-6** was consumed completely. The reaction mixture was quenched by addition NH_4Cl (1.00 L x 2) at 25°C, and then diluted with ethyl acetate (500 mL) and washed with water (500 mL). The combined organic layers were washed with brine (500 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate/TEA = 10/1/0.5% to 0/1/0.5%). Compound **26-7** (34.0 g, 47.1 mmol, 62.5% yield) was obtained as a colorless oil.

^{1}H NMR: 400 MHz DMSO-d_6 δ ppm 11.40 (d, J = 1.8 Hz, 1H), 7.68 (d, J = 8.2 Hz, 1H), 7.24-7.33 (m, 5H), 7.14-7.22 (m, 5H), 6.81-6.88 (m, 4H), 5.96-6.02 (m, 1H), 5.55 (dd, J = 8.0, 2.0 Hz, 1H), 3.73 (d, J = 0.8 Hz, 6H), 3.70 (dd, J = 6.0., 2.2 Hz, 2H), 3.62-3.66 (m, 2H), 3.53-3.57 (m, 2H), 3.50-3.53 (m, 1H), 3.38-3.42 (m, 2H), 3.20-3.22 (m, 3H), 2.94-3.00 (m, 2H), 0.72-0.82 (m, 9H), -0.04 (d, J = 7.4 Hz, 5H)

[0461] Compound 26-8



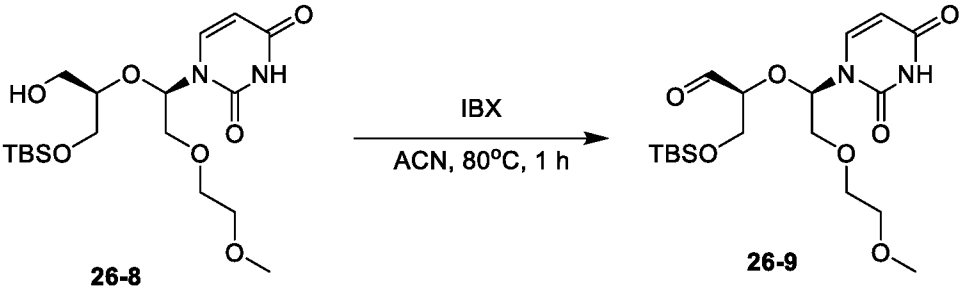
26-7 **26-8**

To a solution of compound **26-7** (46.8 g, 64.9 mmol, 1.00 eq) in DCM (468 mL) was added TFA (43.1 g, 378 mmol, 28.0 mL, 5.83 eq) and Et_3SiH (15.1 g, 129 mmol, 20.7 mL, 2.00 eq). The mixture was stirred at 25°C for 1 hr. TLC (Petroleum ether/Ethyl acetate = 0/1, compound **26-8**: R_f = 0.43) indicated compound **26-7** was consumed completely. The reaction mixture was diluted with DCM (500 mL) and washed with H_2O (500 mL). The combined organic layers were washed with $NaHCO_3$ (500 mL x 3) and brine (500 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 10/1 to 0/1). Compound **26-8** (17.0 g, 36.5 mmol, 56.3% yield, 90.0% purity) was obtained as a colorless oil.

^{1}H NMR: 400 MHz, DMSO-d_6 δ ppm 11.87 (s, 1H), 8.17 (d, J = 8.2 Hz, 1H), 7.75-7.87 (m, 4H), 7.63-7.74 (m, 5H), 7.36 (dd, J = 9.0, 2.6 Hz, 4H), 6.34 (t, J = 5.8 Hz, 1H), 6.05 (d, J = 8.0

Hz, 1H), 5.62 (t, J = 5.8 Hz, 1H), 4.24 (s, 6H), 4.09-4.20 (m, 4H), 4.02 (d, J = 4.6 Hz, 1H), 3.83 (s, 1H), 2.95-3.09 (m, 4H), 1.28 (s, 9H), 0.48 (d, J = 8.2 Hz, 6H).

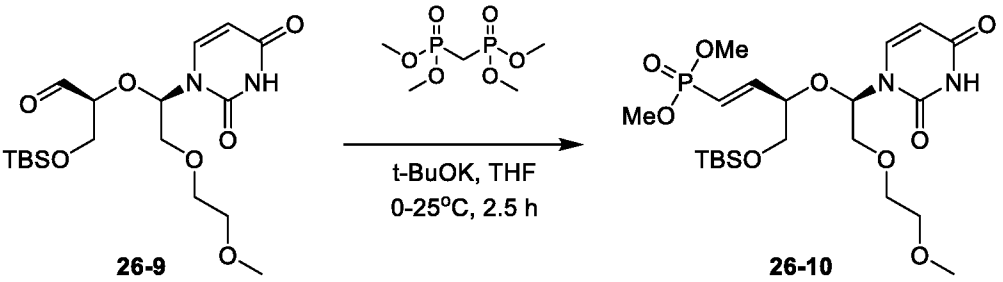
[0462] Compound 26-9



26-8 **26-9**

To a solution of compound **26-8** (17.0 g, 40.6 mmol, 1.00 eq) in ACN (170 mL) was added IBX (17.1 g, 60.9 mmol, 1.50 eq). The mixture was stirred at 80°C for 1 hr. LC-MS showed compound **26-8** was consumed completely. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product compound **26-9** (17.0 g, crude) was obtained as a colorless oil and used in the next step without further purification.

[0463] Compound 26-10

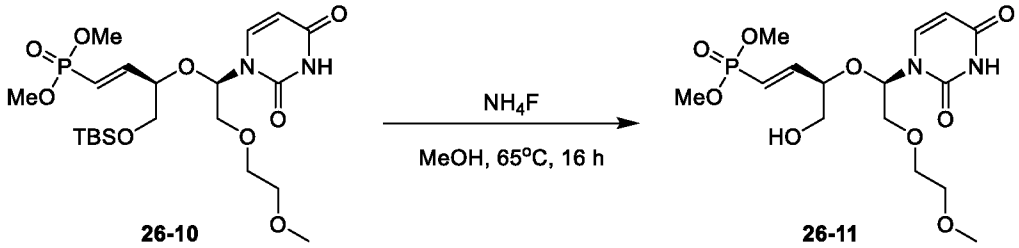


26-9 **26-10**

To a solution of compound tetramethyl methylenediphosphonate (15.1 g, 65.3 mmol, 1.60 eq) in THF (100 mL) was added dropwise t-BuOK (6.87 g, 61.2 mmol, 1.50 eq) at 0°C,and the mixture stirred at 25°C for 0.5 hr. The mixture was then added dropwise to compound **26-9** (17.0 g, 40.8 mmol, 1.00 eq) in THF (70.0 mL) and stirred at 0°C for 1 h, allowed to reach 25°C and stirred at 25°C for 1 h. TLC (Petroleum ether/Ethyl acetate = 0/1, compound 10: R_f = 0.07) indicated compound **26-9** was consumed completely. The reaction mixture was quenched by addition NH_4Cl (500 mL), and then diluted with ethyl acetate (200 mL) and extracted. The combined organic layers were washed with brine (200 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate=10/1 to 0/1). Compound **26-10** (11.4 g, 16.3 mmol, 40.1% yield, 75.0% purity) was obtained as a white solid.

^{1}H NMR: 400 MHz, $CDCl_3$ δ ppm 9.19 (s, 1H), 7.43 (d, *J* = 8.16 Hz, 1H), 6.51-6.67 (m, 1H), 6.13 (t, *J* = 4.78 Hz, 1H), 5.82-5.95 (m, 1H), 5.72 (dd, *J* = 8.04, 1.88 Hz, 1H), 4.20 (s, 1H), 3.63-3.78 (m, 12H), 3.45-3.54 (m, 2H), 3.31-3.37 (m, 3H), 0.82-0.94 (m, 9H), 0.00-0.10 (m, 6H).

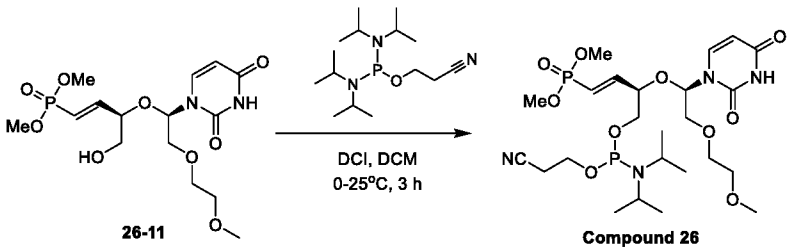
[0464] Compound 26-11



To a solution of compound **26-10** (11.4 g, 21. mmol, 1.00 eq) in methanol (114 mL) was added NH_4F (6.46 g, 174 mmol, 8.00 eq). The mixture was stirred at 65°C for 16 h. TLC (DCM/MeOH = 20/1, compound 10: R_f = 0.28) indicated compound **26-10** was consumed completely. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Ethyl acetate/MeOH = 100/1 to 10/1). Compound **26-11** (5.90 g, 13.0 mmol, 59.6% yield, 90.0% purity) was obtained as a colorless oil.

^{1}H NMR: 400 MHz DMSO-d_6, δ ppm 11.29 (s, 1H) 7.74 (d, *J* = 8.0 Hz, 1H) 6.42-6.62 (m, 1H) 5.90-5.98 (m, 2H) 5.62 (dd, *J* = 8.0, 1.2 Hz, 1H) 5.01 (t, *J* = 5.6 Hz, 1H) 4.18 (d, *J* = 1.6 Hz, 1H) 4.10 (q, *J* = 5.2 Hz, 1H) 3.70-3.76 (m, 2H) 3.50-3.58 (m, 8H) 3.43-3.49 (m, 1H) 3.36-3.41 (m, 2H) 3.21 (s, 3H) 3.17 (d, *J* = 5.2 Hz, 2H) 1.91 (s, 1H)

Compound 26



To a solution of compound **26-11** (4.90 g, 12.0 mmol, 1.00 eq) in DCM (49.0 mL) was added DCI (2.27 g, 19.2 mmol, 1.60 eq) and 2-Cyanoethyl N,N,N',N-tetraisopropylphosphorodiamidite (6.51 g, 21.6 mmol, 6.86 mL, 1.80 eq) at 0°C. The mixture was stirred at 25°C for 3 hrs. TLC (DCM/MeOH = 10/1, Compound **26**: R_f = 0.58) indicated compound **26-11** was consumed completely. The reaction mixture was diluted with DCM (50.0 mL) and washed with aq. $NaHCO_3$ (50.0 mL x 2). The combined organic layers were washed with brine (50.0 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to

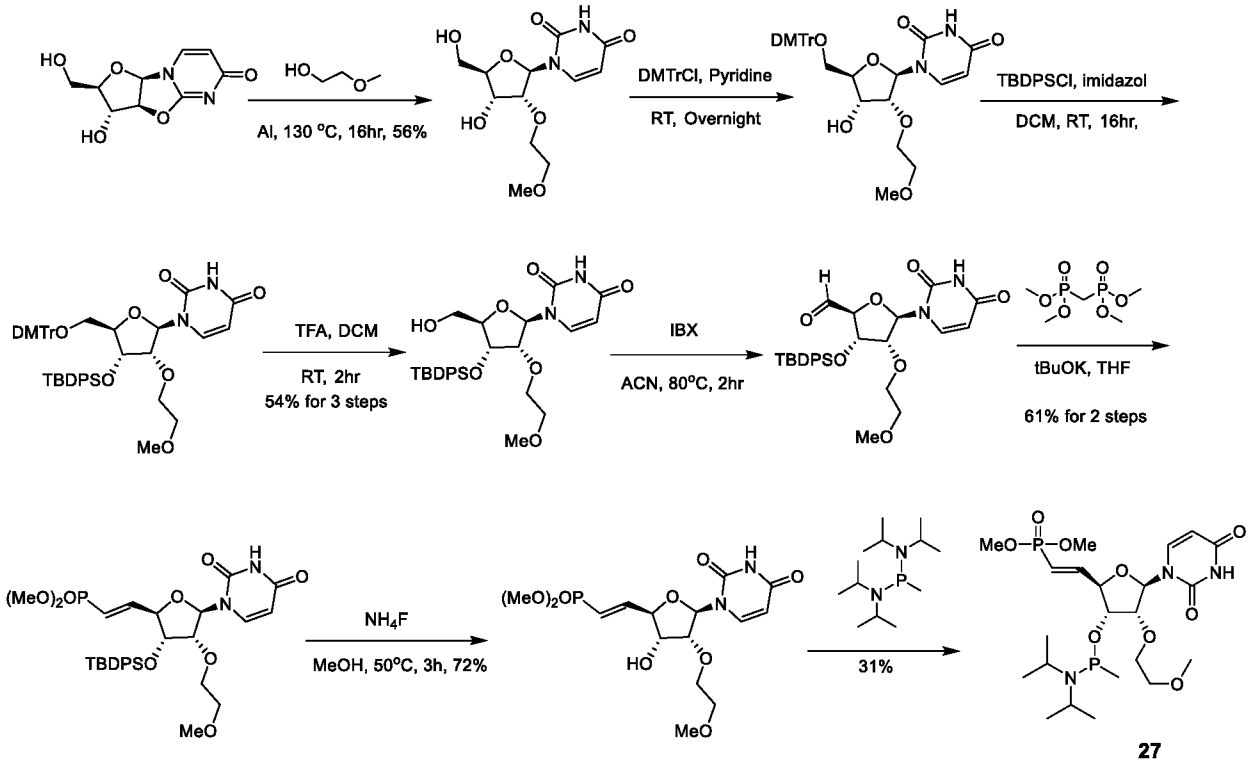
give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate/TEA = 100/1/0.5% to 0/1/0.5%). **Compound 26** (5.40 g, 8.52 mmol, 70.9% yield, 96.0% purity) was obtained as a colorless oil.

^{31}P decoupled ^{1}H NMR: (400MHz, CD_3CN): δ 7.42-7.45(dd, J =8Hz,1H), δ 6.42-6.47(dd, J=17.2 Hz, 1H), 5.97-5.84(m, 2H), 5.54-5.52(d, J=8Hz, 1H), 4.27-4.23(m, 1H), 3.75-3.50(br, 12 H), 3.36-3.30(br, 2H), 3.18(s, 3H), 2.60-2.57(m, 2H), 1.10-1.09(d, 12H)

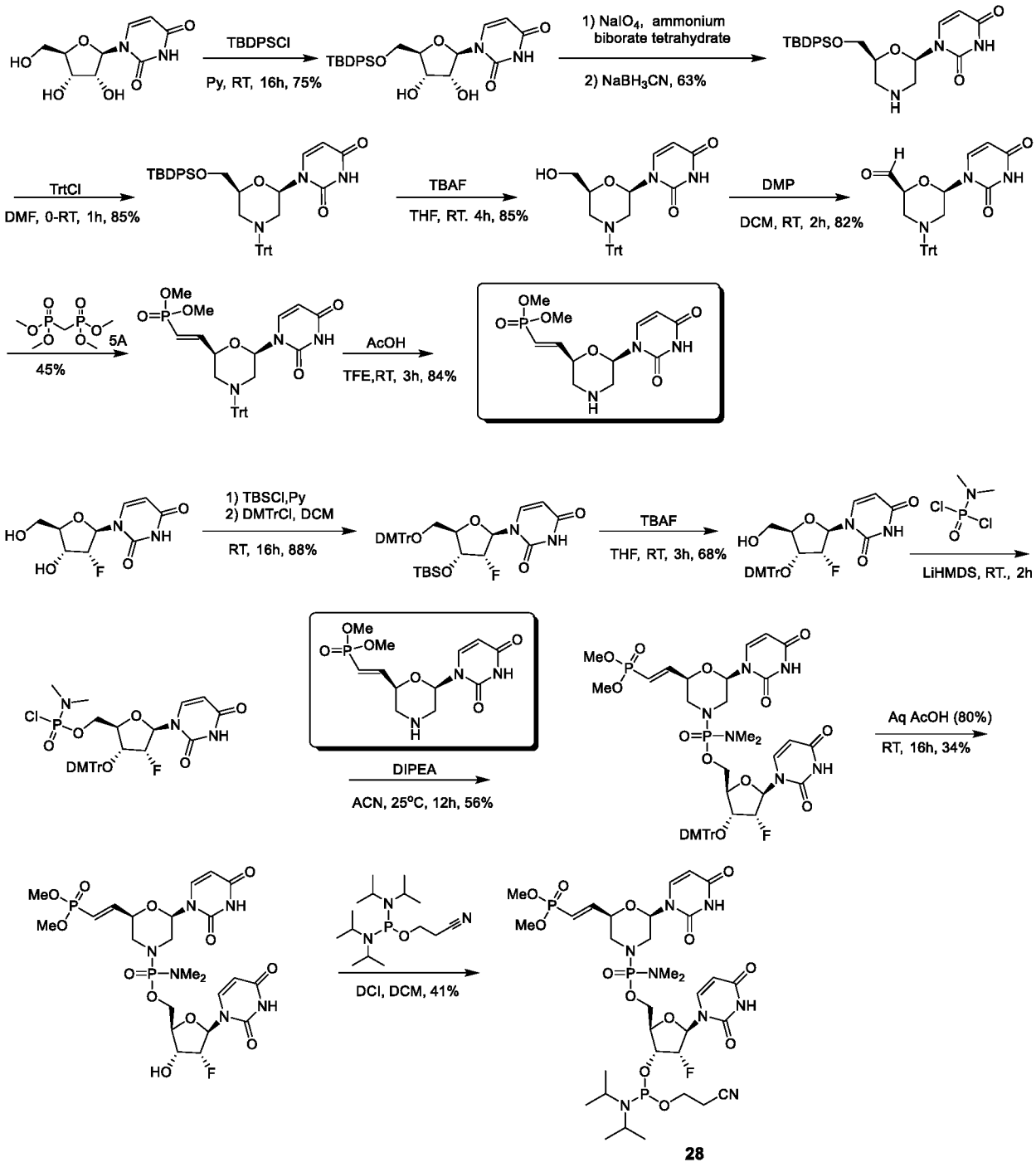
^{31}P NMR (400 MHz, CD_3CN) δ148.6, 148.4, 19.24, 19.22

MS (ESI) calculated for $C_{24}H_{42}N_4O_{10}P_2$ $(M-H)^-$ m/z = 607.2, found 607.2

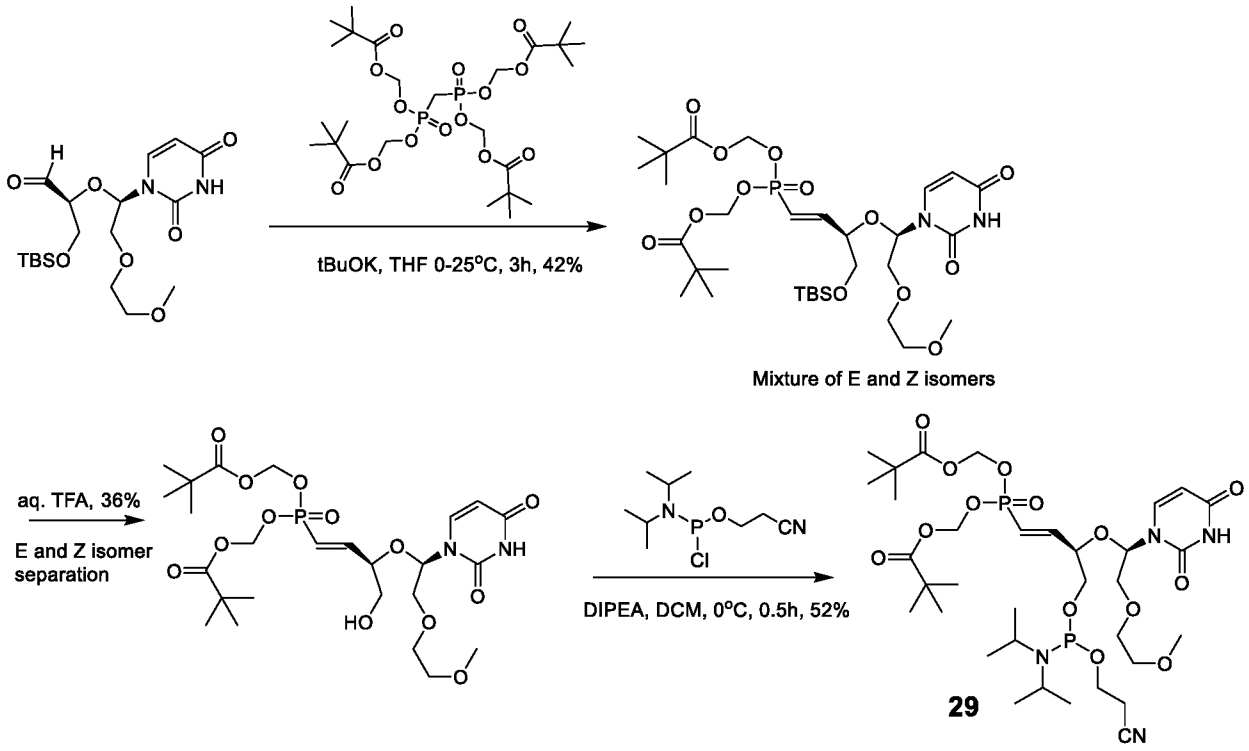
[0465] Example 20. Preparation of Compound 27



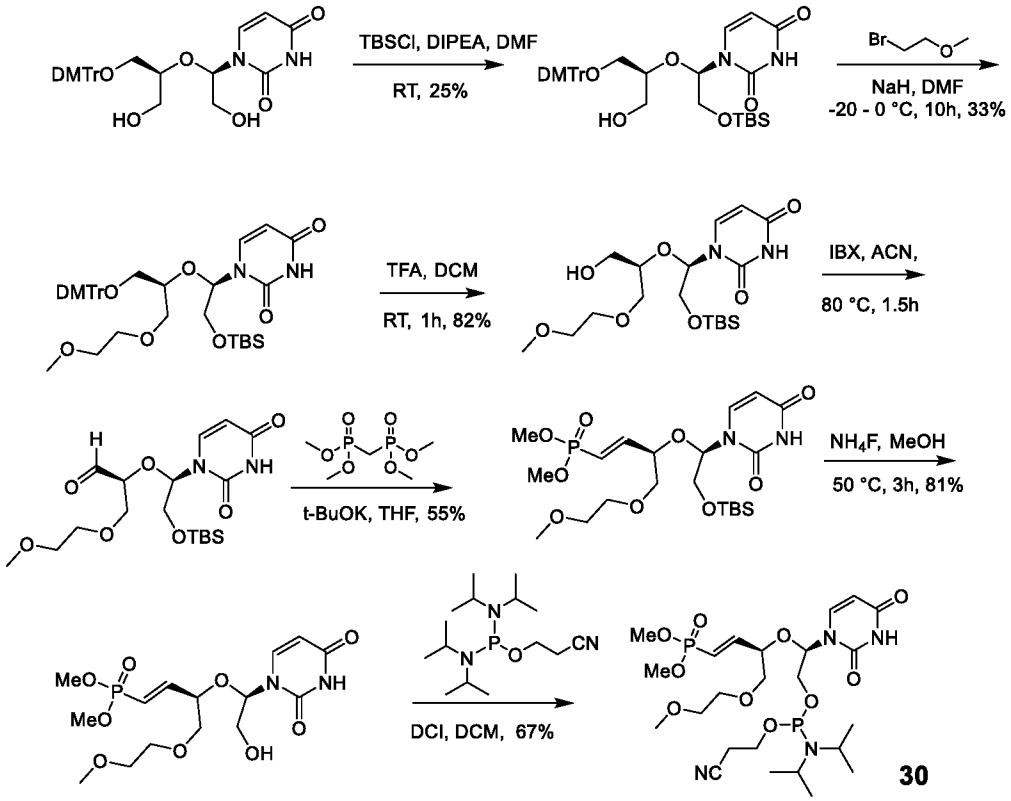
[0466] Example 21. Preparation of Compound 28



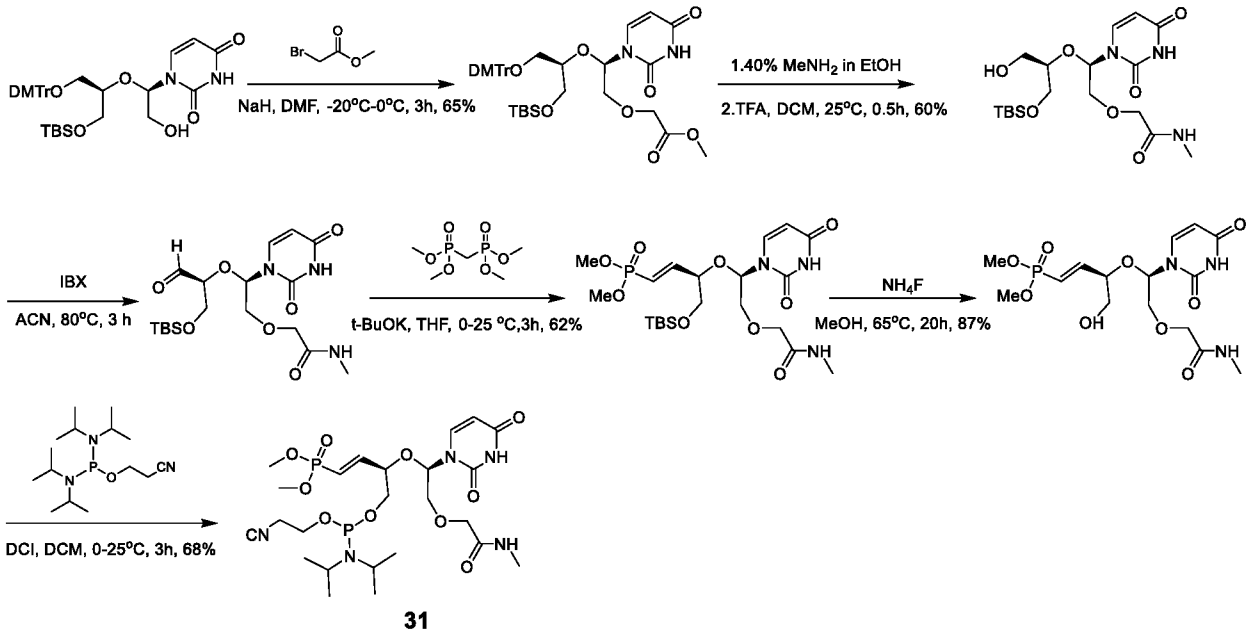
[0467] Example 22. Preparation of Compound 29



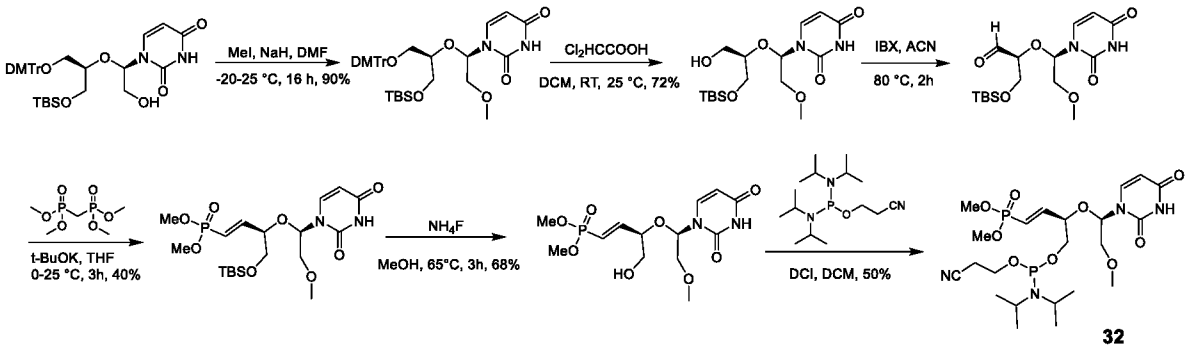
[0468] Example 23. Preparation of Compound 30



[0469] Example 24. Preparation of Compound 31

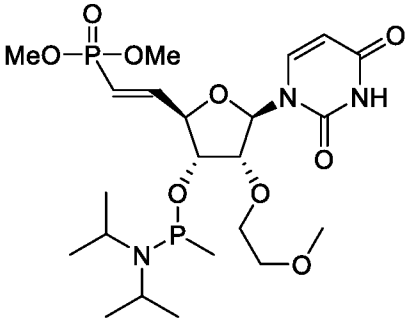


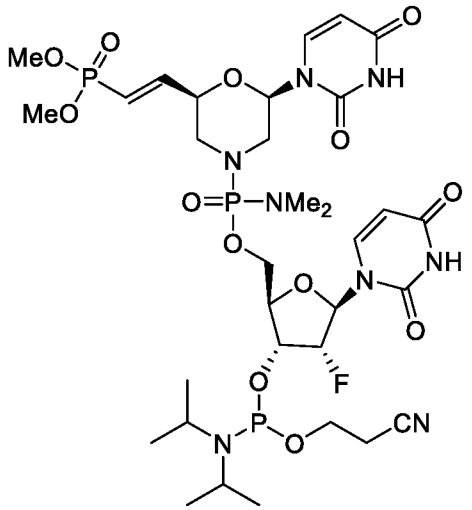
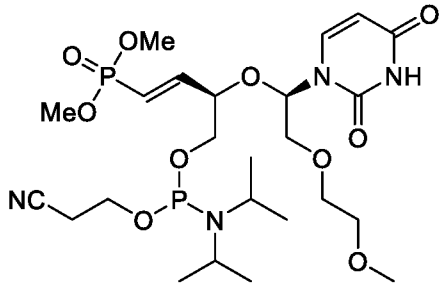
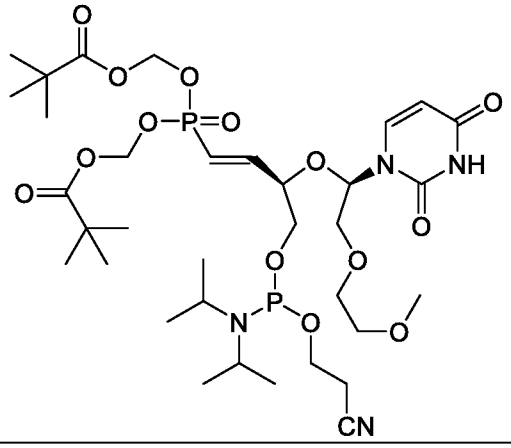
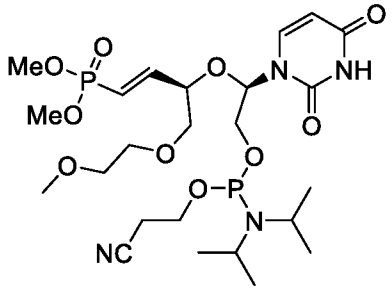
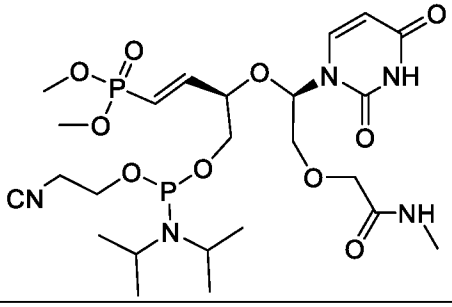
[0470] Example 25. Preparation of Compound 32

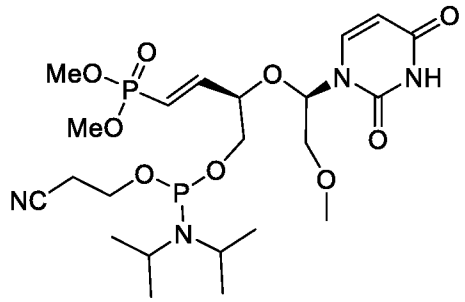
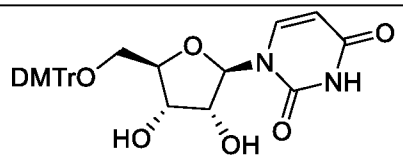
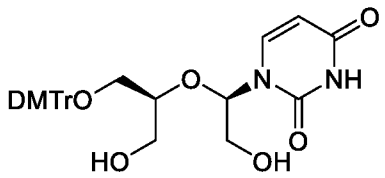
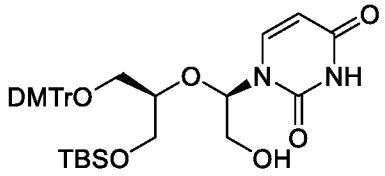
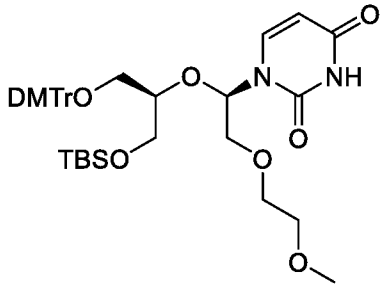
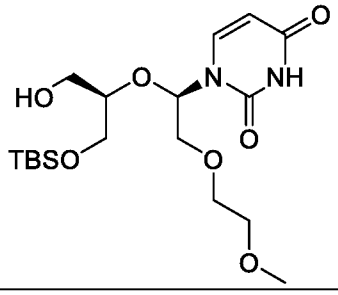


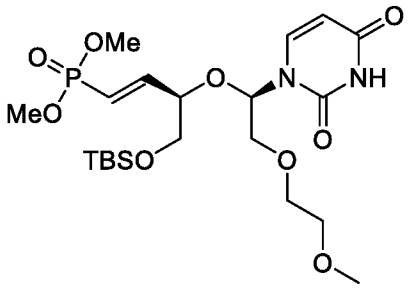
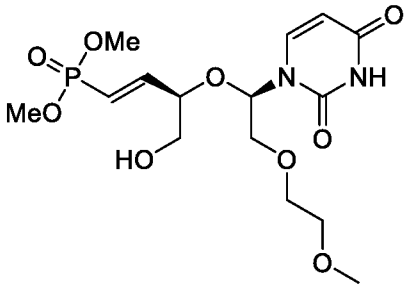
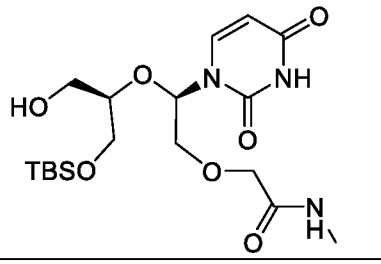
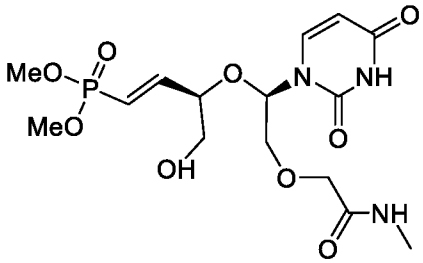
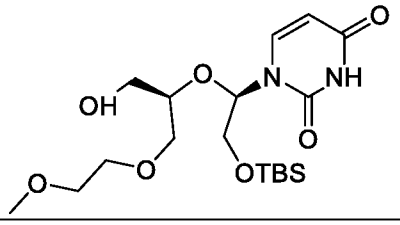
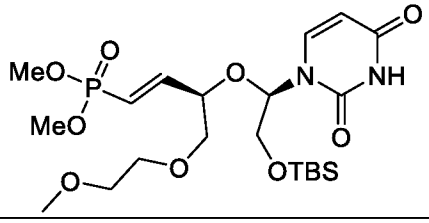
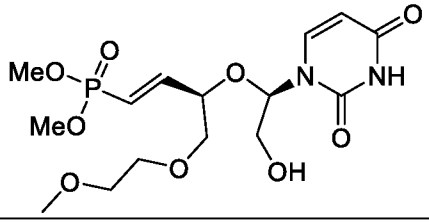
[0471] Example 26. Analytical data

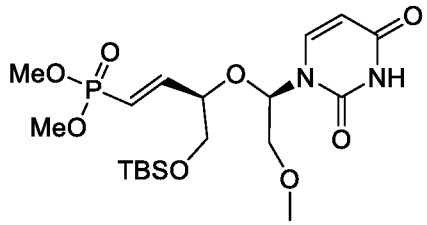
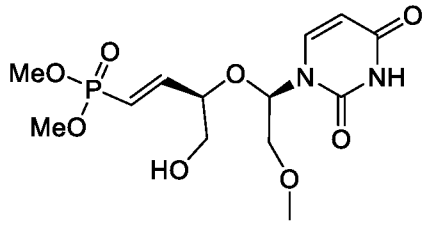
^{31}P decoupled ^{1}H NMR, ^{31}P NMR and MS (ESI) data for various of the compounds described herein are pressented below:

Chemical structure	Analytical (NMR and MS) data
	^{31}P decoupled ^{1}H NMR (400MHz, CD_3CN): δ 7.39-7.35(dd,J =7.6Hz,1H), δ6.77(m,1H), 6.0(m,1H), 5.82-5.78 (dd,J=3.6,14Hz,1H), 5.66-5.63(dd, J=4, 8Hz,1H), 4.52-4.46(m,1H), 4.16-4.01(m, 2H), 3.73-3.47(br,12 H), 3.28(s,3H), 1.26(d,J=6.4Hz,3H), 1.18-1.16(d,J=6.4Hz, 6H), 1.09-1.08(d,J=6.4Hz,6H) ^{31}P NMR (400 MHz, CD_3CN) δ125.7, 125.5, 19.09, 18.95 MS (ESI) calculated for $C_{24}H_{42}N_4O_{10}P_2$ (M+H)+ m/z = 652.2, found 652.2

	^{31}P decoupled ^{1}H NMR (400MHz, CD_3CN): δ7.70-7.61(m,1H), δ7.55-7.48(m, 1H), 7.08-6.73(br, 1H), 6.01(br, 1H), 5.69(br, 4H), 5.3 (m, 1H), 4.78-4.49(m, 2H), 4.11(br, 3H), 3.90-3.48(br,14H), 2.83-2.59(Br,10H), 1.35-1.20(m,12H) ^{31}P NMR (400 MHz, CD_3CN) δ150.75, 150.71, 150.05, 149.99, 20.54, 20.44, 15.39, 15.29 MS (ESI) calculated for $C_{32}H_{50}FN_8O_{13}P_3$ $(M-H)^-$ m/z = 865.2, found 865.2
	^{31}P decoupled ^{1}H NMR (400MHz, CD_3CN): δ 7.42-7.45(dd, J =8Hz,1H), δ 6.42-6.47(dd, J=17.2 Hz, 1H), 5.97-5.84(m, 2H), 5.54-5.52(d, J=8Hz, 1H), 4.27-4.23(m, 1H), 3.75-3.50(br, 12 H), 3.36-3.30(br, 2H), 3.18(s, 3H), 2.60-2.57(m, 2H), 1.10-1.09(d, 12H) ^{31}P NMR (400 MHz, CD_3CN) δ148.6, 148.4, 19.24, 19.22 MS (ESI) calculated for $C_{24}H_{42}N_4O_{10}P_2$ $(M-H)^-$ m/z = 607.2, found 607.2
	^{31}P decoupled ^{1}H NMR (400MHz, CD_3CN): δ 7.55 (dd, J =8Hz,1H), δ 6.58-6.69 (m, J=17.2 Hz, 1H), 6.11-6.02 (m, 2H), 5.66-5.47 (m, 5H), 4.36-4.38 (m, 1H), 3.88-3.60 (m, 9 H), 3.46-3.44 (br, 2H), 3.29 (s, 3H), 2.69 (dt, J=1.6Hz, 6Hz, 2H), 1.20 (m, 30H) ^{31}P NMR (400 MHz, CD_3CN) δ148.69, 148.49, 16.79 MS (ESI) calculated for $C_{34}H_{58}N_4O_{14}P_2$ $(M-H)^-$ m/z = 807.3, found 807.3
	^{31}P decoupled ^{1}H NMR (400MHz, CD_3CN): δ 7.55-7.52(dd, J =8Hz,1H), δ 6.58-6.52(dd, J=17.2 Hz, 1H), 6.08-5.94(m, 2H), 5.67-5.64(d, J=8Hz, 1H), 4.39-4.35(m, 1H), 3.95-3.79(br, 4 H),3.75-3.58 (br, 12H), 3.57-3.49(br, 2H), 3.33(br, 3H), 2.68-2.65(t, 2H), 1.18-1.13(m, 12H) ^{31}P NMR (400 MHz, CD_3CN) δ149.05, 148.82, 19.41, 19.38 MS (ESI) calculated for $C_{24}H_{42}N_4O_{10}P_2$ $(M-H)^-$ m/z = 607.2, found 607.2
	^{1}H NMR (400MHz, CD_3CN): δ7.55 (d, J=8Hz,1H), 6.83 (br, 1H) δ6.57 (ddd, J=5.2, 17.2, 39.2 Hz, 1H), 6.12-5.95 (m, 2H), 5.68(d, J=8Hz, 1H), 4.4 (br, 1H), 3.96 (s, 2H), 3.88-3.52 (br, 14H), 2.35-2.68 (br, 5H), 1.20 (d, J=6.8Hz, 12H) ^{31}P NMR (400 MHz, CD_3CN) δ148.6, 148.4, 19.28 MS (ESI) calculated for chemical formula: $C_{24}H_{41}N_5O_{10}P_2$ $(M-H)^-$ m/z = 620.2, found 620.2

	^{1}H NMR (400MHz, CD_3CN): δ9.02, (br,1H), δ7.51 (d, J=8Hz,1H), 6.53 (br, 1H) δ6.06 (br, 2H), 5.68(d, J=8Hz, 1H), 4.37 (m,1H), 3.87-3.7 (m, 4H), 3.69-3.63 (m, 10H), 3.35 (s, 3H), 2.70 (m, 2H), 1.22 (m, 12H) ^{31}P NMR (400 MHz, CD_3CN) δ148.7, 148.4, 19.19, 19.15 MS (ESI) calculated for chemical formula: $C_{22}H_{38}N_4O_9P_2$ $(M-H)^-$ m/z = 563.2, found 563.2
	^{1}H NMR: 400 MHz, DMSO-d_6 δ ppm 11.35 (s, 1 H), 7.72 (d, J = 8.0 Hz, 1H), 7.36-7.41 (m, 2H), 7.32 (t, J = 7.6 Hz, 2H), 7.20-7.28 (m, 5H), 6.90 (d, J = 8.6 Hz, 4H), 5.48 (d, J = 4.8 Hz, 1H), 5.31 (d, J = 8.0 Hz, 1H), 5.14 (d, J = 5.6 Hz, 1H), 4.09 (q, J = 5.4 Hz, 2H), 3.92-3.99 (m, 1H), 3.70-3.78 (m, 6H), 3.18-3.30 (m, 2H).
	^{1}H NMR: 400 MHz, DMSO-d_6 δ ppm 11.35 (s, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.28-7.33 (m, 4H), 7.17-7.21 (m, 5H), 6.86 (d, J = 8.0 Hz, 4H), 5.83 (t, J = 6.4 Hz, 1H), 5.53 (d, J = 8.0 Hz, 1H), 5.13 (t, J = 8.0 Hz, 1H), 4.75 (t, J = 5.2 Hz, 1H), 3.76 (s, 6H), 3.68-3.73 (m, 1H), 3.60-3.64 (m, 2H), 3.42 (t, J = 5.2 Hz, 2H), 2.96-3.02 (m, 2H)
	^{1}H NMR: 400 MHz, DMSO-d_6 δ ppm 11.35 (s, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.24-7.33 (m, 4H), 7.14-7.22 (m, 5H), 6.82-6.88 (m, 4H), 5.83 (t, J = 5.8 Hz, 1H), 5.47-5.57 (m, 1H), 5.11 (t, J = 5.8 Hz, 1H), 3.72 (d, J = 0.8 Hz, 6H), 3.57-3.67 (m, 4H), 3.46-3.55 (m, 1H), 2.94-3.00 (m, 2H), 0.72-0.78 (m, 9H), -0.04 (d, J = 8.2 Hz, 5H)
	^{1}H NMR: 400 MHz, DMSO-d_6 δ ppm 11.40 (d, J = 1.8 Hz, 1H), 7.68 (d, J = 8.2 Hz, 1H), 7.24-7.33 (m, 5H), 7.14-7.22 (m, 5H), 6.81-6.88 (m, 4H), 5.96-6.02 (m, 1H), 5.55 (dd, J = 8.0, 2.0 Hz, 1H), 3.73 (d, J = 0.8 Hz, 6H), 3.70 (dd, J = 6.0., 2.2 Hz, 2H), 3.62-3.66 (m, 2H), 3.53-3.57 (m, 2H), 3.50-3.53 (m, 1H), 3.38-3.42 (m, 2H), 3.20-3.22 (m, 3H), 2.94-3.00 (m, 2H), 0.72-0.82 (m, 9H), -0.04 (d, J = 7.4 Hz, 5H)
	^{1}H NMR: 400 MHz, DMSO-d_6 δ ppm 11.87 (s, 1H), 8.17 (d, J = 8.2 Hz, 1H), 7.75-7.87 (m, 4H), 7.63-7.74 (m, 5H), 7.36 (dd, J = 9.0, 2.6 Hz, 4H), 6.34 (t, J = 5.8 Hz, 1H), 6.05 (d, J = 8.0 Hz, 1H), 5.62 (t, J = 5.8 Hz, 1H), 4.24 (s, 6H), 4.09-4.20 (m, 4H), 4.02 (d, J = 4.6 Hz, 1H), 3.83 (s, 1H), 2.95-3.09 (m, 4H), 1.28 (s, 9H), 0.48 (d, J = 8.2 Hz, 6H).

	^{1}H NMR: 400 MHz, $CDCl_3$ δ ppm 9.19 (s, 1H), 7.43 (d, *J* = 8.16 Hz, 1H), 6.51-6.67 (m, 1H), 6.13 (t, *J* = 4.78 Hz, 1H), 5.82-5.95 (m, 1H), 5.72 (dd, *J* = 8.04, 1.88 Hz, 1H), 4.20 (s, 1H), 3.63-3.78 (m, 12H), 3.45-3.54 (m, 2H), 3.31-3.37 (m, 3H), 0.82-0.94 (m, 9H), 0.00-0.10 (m, 6H).
	^{1}H NMR: 400 MHz, DMSO-d_6 δ ppm 11.29 (s, 1H) 7.74 (d, *J* = 8.0 Hz, 1H) 6.42-6.62 (m, 1H) 5.90-5.98 (m, 2H) 5.62 (dd, *J* = 8.0, 1.2 Hz, 1H) 5.01 (t, *J* = 5.6 Hz, 1H) 4.18 (d, *J* = 1.6 Hz, 1H) 4.10 (q, *J* = 5.2 Hz, 1H) 3.70-3.76 (m, 2H) 3.50-3.58 (m, 8H) 3.43-3.49 (m, 1H) 3.36-3.41 (m, 2H) 3.21 (s, 3H) 3.17 (d, *J* = 5.2 Hz, 2H) 1.91 (s, 1H)
	^{1}H NMR: 400 MHz, DMSO-d_6 δ 11.30 (d, *J* = 1.8 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 7.61 (br d, *J* = 4.3 Hz, 1H), 6.03 (t, *J* = 5.6 Hz, 1H), 5.61 (dd, *J* = 2.0, 8.0 Hz, 1H), 4.66 (br s, 1H), 3.90 (s, 2H), 3.73-3.57 (m, 4H), 3.49-3.43 (m, 1H), 3.34-3.25 (m, 2H), 2.60 (d, *J* = 4.8 Hz, 3H), 0.87 (s, 9H), 0.05 (s, 6H)
	^{1}HNMR: 400 MHz CD_3CN δ 7.52 (d, *J* = 8.2 Hz, 1H), 6.98 (br s, 1H), 6.61-6.47 (m, 1H), 6.06-5.86 (m, 2H), 5.65 (d, *J* = 8.2 Hz, 1H), 4.28-4.20 (m, 1H), 3.96 (s, 2H), 3.80-3.51 (m, 10H), 2.73-2.67 (m, 4H) ^{31}PNMR: 162 MHz CD_3CN, δ 19.71 (s, 1P)
	^{1}H NMR: 400 MHz, DMSO d6 11.23 (s, 1H), 7.63 (d, *J* = 8.02 Hz, 1H), 5.85 (dd, *J* = 6.56, 4.98 Hz, 1H), 5.60 (dd, *J* = 7.92, 1.66 Hz, 1H), 4.65 (t, *J* = 5.58 Hz, 1H), 3.63-3.84 (m, 2H), 3.40-3.60 (m, 7H), 3.26 (s, 3H), 0.81 (s, 9H), 0.01 (d, *J* = 6.66 Hz, 5H).
	^{1}H NMR: 400 MHz, DMSO d6 7.72 (d, *J* = 8.22 Hz, 1H), 6.38-6.61 (m, 1H), 5.76-6.09 (m, 2H), 5.61 (dd, *J* = 8.02, 2.16 Hz, 1H), 4.29-4.43 (m, 1H), 3.66-3.92 (m, 2H), 3.41-3.63 (m, 12H), 3.17-3.26 (m, 3H), 0.73-0.87 (m, 10H), 0.01 (d, *J* = 7.04 Hz, 7H).
	^{1}H NMR: 400 MHz, DMSO d6 11.26 (d, *J* = 1.52 Hz, 1H), 7.71 (d, *J* = 8.04 Hz, 1H), 6.43-6.58 (m, 1H), 5.88-6.06 (m, 1H), 5.75-5.85 (m, 1H), 5.61 (dd, *J* = 7.96, 2.08 Hz, 1H), 5.14 (t, *J* = 6.02 Hz, 1H), 4.34 (br d, *J* = 1.52 Hz, 1H), 3.42-3.70 (m, 14H), 3.26 (s, 3H)

	^{1}H NMR: 400 MHz, DMSO-d_6 11.31 (s, 1H), 7.72-7.77 (m, 1H), 6.45-6.56 (m, 1H), 5.91-6.03 (m, 2H), 5.62 (dd, J = 8.40, 2.40 Hz, 1 H), 4.24 (br s, 1H), 3.72-3.76 (m, 1 H), 3.61-3.69 (m, 3H), 3.52-3.59 (m, 6 H), 3.26 (s, 3H), 0.87 (s, 9H), 0.06 (d, J = 1.60 Hz, 6H). ^{31}P NMR: 162 MHz, DMSO-d_6 19.93 (s, 1P)
	^{1}H NMR: 400 MHz, DMSO-d_6 11.31 (s, 1H), 7.74 (d, J = 8.00 Hz, 1H), 6.47-6.58 (m, 1H), 5.87-5.99 (m, 2H), 5.63 (d, J = 8.4 Hz, 1H), 5.04 (s, 1H), 4.10-4.19 (m, 2H), 3.66 (d, J = 6.00 Hz, 2H), 3.56 (dd, J = 11.2, 4.40 Hz, 6H), 3.43-3.48 (m, 1H), 3.28 (s, 3H), 3.17-3.18 (m, 2H). ^{31}P NMR: 162 MHz, DMSO-d_6 20.04 (s, 1P).

Molecular Biology Examples

Example 1. Sequences

[0472] Tables 1, 3, 5, 6, and 7 illustrate target sequences described herein. Tables 2, 4, 8, and 9 illustrate polynucleic acid molecule sequences described herein.

Table 1. KRAS Target Sequences

Id #	sequence position in NM_033360.2	target site in NM_033360.2	SEQ ID NO:
182	182-200	AAAUGACUGAAUAUAAACUUGUG	1
183	183-201	AAUGACUGAAUAUAAACUUGUGG	2
197	197-215	AACUUGUGGUAGUUGGAGCUGGU	3
224	224-242	UAGGCAAGAGUGCCUUGACGAUA	4
226	226-244	GGCAAGAGUGCCUUGACGAUACA	5
227	227-245	GCAAGAGUGCCUUGACGAUACAG	6
228	228-246	CAAGAGUGCCUUGACGAUACAGC	7
232	232-250	AGUGCCUUGACGAUACAGCUAAU	8
233	233-251	GUGCCUUGACGAUACAGCUAAUU	9
236	236-254	CCUUGACGAUACAGCUAAUUCAG	10
237	237-255	CUUGACGAUACAGCUAAUUCAGA	11
245	245-263	UACAGCUAAUUCAGAAUCAUUUU	12
266	266-284	UUGUGGACGAAUAUGAUCCAACA	13
269	269-287	UGGACGAAUAUGAUCCAACAAUA	14
270	270-288	GGACGAAUAUGAUCCAACAAUAG	15

Table 2. KRAS siRNA sequences

Id #	sequence position in	sense strand sequence (5′-3′)	SEQ ID	antisense strand sequence (5′-3′)	SEQ ID

	NM_033360.2		NO:		NO:
182	182-200	AUGACUGAAUAUAAACUUGTT	16	CAAGUUUAUAUUCAGUCAUTT	17
183	183-201	UGACUGAAUAUAAACUUGUTT	18	ACAAGUUUAUAUUCAGUCATT	19
197	197-215	CUUGUGGUAGUUGGAGCUGTT	20	CAGCUCCAACUACCACAAGTT	21
224	224-242	GGCAAGAGUGCCUUGACGATT	22	UCGUCAAGGCACUCUUGCCTT	23
226	226-244	CAAGAGUGCCUUGACGAUATT	24	UAUCGUCAAGGCACUCUUGTT	25
227	227-245	AAGAGUGCCUUGACGAUACTT	26	GUAUCGUCAAGGCACUCUUTT	27
228	228-246	AGAGUGCCUUGACGAUACATT	28	UGUAUCGUCAAGGCACUCUTT	29
232	232-250	UGCCUUGACGAUACAGCUATT	30	UAGCUGUAUCGUCAAGGCATT	31
233	233-251	GCCUUGACGAUACAGCUAATT	32	UUAGCUGUAUCGUCAAGGCTT	33
236	236-254	UUGACGAUACAGCUAAUUCTT	34	GAAUUAGCUGUAUCGUCAATT	35
237	237-255	UGACGAUACAGCUAAUUCATT	36	UGAAUUAGCUGUAUCGUCATT	37
245	245-263	CAGCUAAUUCAGAAUCAUUTT	38	AAUGAUUCUGAAUUAGCUGTT	39
266	266-284	GUGGACGAAUAUGAUCCAATT	40	UUGGAUCAUAUUCGUCCACTT	41
269	269-287	GACGAAUAUGAUCCAACAATT	42	UUGUUGGAUCAUAUUCGUCTT	43
270	270-288	ACGAAUAUGAUCCAACAAUTT	44	AUUGUUGGAUCAUAUUCGUTT	45

Table 3. EGFR Target Sequences

hs Id #	19mer pos. in NM_005228.3	sequence of total 23mer target site in NM_005228.3	SEQ ID NO:
68	68-86	GGCGGCCGGAGUCCCGAGCUAGC	46
71	71-89	GGCCGGAGUCCCGAGCUAGCCCC	47
72	72-90	GCCGGAGUCCCGAGCUAGCCCCG	48
73	73-91	CCGGAGUCCCGAGCUAGCCCCGG	49
74	74-92	CGGAGUCCCGAGCUAGCCCCGGC	50
75	75-93	GGAGUCCCGAGCUAGCCCCGGCG	51
76	76-94	GAGUCCCGAGCUAGCCCCGGCGG	52
78	78-96	GUCCCGAGCUAGCCCCGGCGGCC	53
114	114-132	CCGGACGACAGGCCACCUCGUCG	54
115	115-133	CGGACGACAGGCCACCUCGUCGG	55
116	116-134	GGACGACAGGCCACCUCGUCGGC	56
117	117-135	GACGACAGGCCACCUCGUCGGCG	57
118	118-136	ACGACAGGCCACCUCGUCGGCGU	58
120	120-138	GACAGGCCACCUCGUCGGCGUCC	59
121	121-139	ACAGGCCACCUCGUCGGCGUCCG	60
122	122-140	CAGGCCACCUCGUCGGCGUCCGC	61
123	123-141	AGGCCACCUCGUCGGCGUCCGCC	62
124	124-142	GGCCACCUCGUCGGCGUCCGCCC	63

125	125-143	GCCACCUCGUCGGCGUCCGCCCG	64
126	126-144	CCACCUCGUCGGCGUCCGCCCGA	65
127	127-145	CACCUCGUCGGCGUCCGCCCGAG	66
128	128-146	ACCUCGUCGGCGUCCGCCCGAGU	67
129	129-147	CCUCGUCGGCGUCCGCCCGAGUC	68
130	130-148	CUCGUCGGCGUCCGCCCGAGUCC	69
131	131-149	UCGUCGGCGUCCGCCCGAGUCCC	70
132	132-150	CGUCGGCGUCCGCCCGAGUCCCC	71
135	135-153	CGGCGUCCGCCCGAGUCCCCGCC	72
136	136-154	GGCGUCCGCCCGAGUCCCCGCCU	73
141	141-159	CCGCCCGAGUCCCCGCCUCGCCG	74
164	164-182	CCAACGCCACAACCACCGCGCAC	75
165	165-183	CAACGCCACAACCACCGCGCACG	76
166	166-184	AACGCCACAACCACCGCGCACGG	77
168	168-186	CGCCACAACCACCGCGCACGGCC	78
169	169-187	GCCACAACCACCGCGCACGGCCC	79
170	170-188	CCACAACCACCGCGCACGGCCCC	80
247	247-265	CGAUGCGACCCUCCGGGACGGCC	81
248	248-266	GAUGCGACCCUCCGGGACGGCCG	82
249	249-267	AUGCGACCCUCCGGGACGGCCGG	83
251	251-269	GCGACCCUCCGGGACGGCCGGGG	84
252	252-270	CGACCCUCCGGGACGGCCGGGGC	85
254	254-272	ACCCUCCGGGACGGCCGGGGCAG	86
329	329-347	AAAGAAAGUUUGCCAAGGCACGA	87
330	330-348	AAGAAAGUUUGCCAAGGCACGAG	88
332	332-350	GAAAGUUUGCCAAGGCACGAGUA	89
333	333-351	AAAGUUUGCCAAGGCACGAGUAA	90
334	334-352	AAGUUUGCCAAGGCACGAGUAAC	91
335	335-353	AGUUUGCCAAGGCACGAGUAACA	92
336	336-354	GUUUGCCAAGGCACGAGUAACAA	93
337	337-355	UUUGCCAAGGCACGAGUAACAAG	94
338	338-356	UUGCCAAGGCACGAGUAACAAGC	95
361	361-379	UCACGCAGUUGGGCACUUUUGAA	96
362	362-380	CACGCAGUUGGGCACUUUUGAAG	97
363	363-381	ACGCAGUUGGGCACUUUUGAAGA	98
364	364-382	CGCAGUUGGGCACUUUUGAAGAU	99
365	365-383	GCAGUUGGGCACUUUUGAAGAUC	100
366	366-384	CAGUUGGGCACUUUUGAAGAUCA	101
367	367-385	AGUUGGGCACUUUUGAAGAUCAU	102
368	368-386	GUUGGGCACUUUUGAAGAUCAUU	103
369	369-387	UUGGGCACUUUUGAAGAUCAUUU	104
377	377-395	UUUUGAAGAUCAUUUUCUCAGCC	105
379	379-397	UUGAAGAUCAUUUUCUCAGCCUC	106
380	380-398	UGAAGAUCAUUUUCUCAGCCUCC	107
385	385-403	AUCAUUUUCUCAGCCUCCAGAGG	108
394	394-412	UCAGCCUCCAGAGGAUGUUCAAU	109
396	396-414	AGCCUCCAGAGGAUGUUCAAUAA	110
397	397-415	GCCUCCAGAGGAUGUUCAAUAAC	111
401	401-419	CCAGAGGAUGUUCAAUAACUGUG	112
403	403-421	AGAGGAUGUUCAAUAACUGUGAG	113
407	407-425	GAUGUUCAAUAACUGUGAGGUGG	114
409	409-427	UGUUCAAUAACUGUGAGGUGGUC	115
410	410-428	GUUCAAUAACUGUGAGGUGGUCC	116

411	411-429	UUCAAUAACUGUGAGGUGGUCCU	117
412	412-430	UCAAUAACUGUGAGGUGGUCCUU	118
413	413-431	CAAUAACUGUGAGGUGGUCCUUG	119
414	414-432	AAUAACUGUGAGGUGGUCCUUGG	120
416	416-434	UAACUGUGAGGUGGUCCUUGGGA	121
418	418-436	ACUGUGAGGUGGUCCUUGGGAAU	122
419	419-437	CUGUGAGGUGGUCCUUGGGAAUU	123
425	425-443	GGUGGUCCUUGGGAAUUUGGAAA	124
431	431-449	CCUUGGGAAUUUGGAAAUUACCU	125
432	432-450	CUUGGGAAUUUGGAAAUUACCUA	126
433	433-451	UUGGGAAUUUGGAAAUUACCUAU	127
434	434-452	UGGGAAUUUGGAAAUUACCUAUG	128
458	458-476	GCAGAGGAAUUAUGAUCUUUCCU	129
459	459-477	CAGAGGAAUUAUGAUCUUUCCUU	130
463	463-481	GGAAUUAUGAUCUUUCCUUCUUA	131
464	464-482	GAAUUAUGAUCUUUCCUUCUUAA	132
466	466-484	AUUAUGAUCUUUCCUUCUUAAAG	133
468	468-486	UAUGAUCUUUCCUUCUUAAAGAC	134
471	471-489	GAUCUUUCCUUCUUAAAGACCAU	135
476	476-494	UUCCUUCUUAAAGACCAUCCAGG	136
477	477-495	UCCUUCUUAAAGACCAUCCAGGA	137
479	479-497	CUUCUUAAAGACCAUCCAGGAGG	138
481	481-499	UCUUAAAGACCAUCCAGGAGGUG	139
482	482-500	CUUAAAGACCAUCCAGGAGGUGG	140
492	492-510	AUCCAGGAGGUGGCUGGUUAUGU	141
493	493-511	UCCAGGAGGUGGCUGGUUAUGUC	142
494	494-512	CCAGGAGGUGGCUGGUUAUGUCC	143
495	495-513	CAGGAGGUGGCUGGUUAUGUCCU	144
496	496-514	AGGAGGUGGCUGGUUAUGUCCUC	145
497	497-515	GGAGGUGGCUGGUUAUGUCCUCA	146
499	499-517	AGGUGGCUGGUUAUGUCCUCAUU	147
520	520-538	UUGCCCUCAACACAGUGGAGCGA	148
542	542-560	AAUUCCUUUGGAAAACCUGCAGA	149
543	543-561	AUUCCUUUGGAAAACCUGCAGAU	150
550	550-568	UGGAAAACCUGCAGAUCAUCAGA	151
551	551-569	GGAAAACCUGCAGAUCAUCAGAG	152
553	553-571	AAAACCUGCAGAUCAUCAGAGGA	153
556	556-574	ACCUGCAGAUCAUCAGAGGAAAU	154
586	586-604	ACGAAAAUUCCUAUGCCUUAGCA	155
587	587-605	CGAAAAUUCCUAUGCCUUAGCAG	156
589	589-607	AAAAUUCCUAUGCCUUAGCAGUC	157
592	592-610	AUUCCUAUGCCUUAGCAGUCUUA	158
593	593-611	UUCCUAUGCCUUAGCAGUCUUAU	159
594	594-612	UCCUAUGCCUUAGCAGUCUUAUC	160
596	596-614	CUAUGCCUUAGCAGUCUUAUCUA	161
597	597-615	UAUGCCUUAGCAGUCUUAUCUAA	162
598	598-616	AUGCCUUAGCAGUCUUAUCUAAC	163
599	599-617	UGCCUUAGCAGUCUUAUCUAACU	164
600	600-618	GCCUUAGCAGUCUUAUCUAACUA	165
601	601-619	CCUUAGCAGUCUUAUCUAACUAU	166
602	602-620	CUUAGCAGUCUUAUCUAACUAUG	167
603	603-621	UUAGCAGUCUUAUCUAACUAUGA	168
604	604-622	UAGCAGUCUUAUCUAACUAUGAU	169

605	605-623	AGCAGUCUUAUCUAACUAUGAUG	170
608	608-626	AGUCUUAUCUAACUAUGAUGCAA	171
609	609-627	GUCUUAUCUAACUAUGAUGCAAA	172
610	610-628	UCUUAUCUAACUAUGAUGCAAAU	173
611	611-629	CUUAUCUAACUAUGAUGCAAAUA	174
612	612-630	UUAUCUAACUAUGAUGCAAAUAA	175
613	613-631	UAUCUAACUAUGAUGCAAAUAAA	176
614	614-632	AUCUAACUAUGAUGCAAAUAAAA	177
616	616-634	CUAACUAUGAUGCAAAUAAAACC	178
622	622-640	AUGAUGCAAAUAAAACCGGACUG	179
623	623-641	UGAUGCAAAUAAAACCGGACUGA	180
624	624-642	GAUGCAAAUAAAACCGGACUGAA	181
626	626-644	UGCAAAUAAAACCGGACUGAAGG	182
627	627-645	GCAAAUAAAACCGGACUGAAGGA	183
628	628-646	CAAAUAAAACCGGACUGAAGGAG	184
630	630-648	AAUAAAACCGGACUGAAGGAGCU	185
631	631-649	AUAAAACCGGACUGAAGGAGCUG	186
632	632-650	UAAAACCGGACUGAAGGAGCUGC	187
633	633-651	AAAACCGGACUGAAGGAGCUGCC	188
644	644-662	GAAGGAGCUGCCCAUGAGAAAUU	189
665	665-683	UUUACAGGAAAUCCUGCAUGGCG	190
668	668-686	ACAGGAAAUCCUGCAUGGCGCCG	191
669	669-687	CAGGAAAUCCUGCAUGGCGCCGU	192
670	670-688	AGGAAAUCCUGCAUGGCGCCGUG	193
671	671-689	GGAAAUCCUGCAUGGCGCCGUGC	194
672	672-690	GAAAUCCUGCAUGGCGCCGUGCG	195
674	674-692	AAUCCUGCAUGGCGCCGUGCGGU	196
676	676-694	UCCUGCAUGGCGCCGUGCGGUUC	197
677	677-695	CCUGCAUGGCGCCGUGCGGUUCA	198
678	678-696	CUGCAUGGCGCCGUGCGGUUCAG	199
680	680-698	GCAUGGCGCCGUGCGGUUCAGCA	200
681	681-699	CAUGGCGCCGUGCGGUUCAGCAA	201
682	682-700	AUGGCGCCGUGCGGUUCAGCAAC	202
683	683-701	UGGCGCCGUGCGGUUCAGCAACA	203
684	684-702	GGCGCCGUGCGGUUCAGCAACAA	204
685	685-703	GCGCCGUGCGGUUCAGCAACAAC	205
686	686-704	CGCCGUGCGGUUCAGCAACAACC	206
688	688-706	CCGUGCGGUUCAGCAACAACCCU	207
690	690-708	GUGCGGUUCAGCAACAACCCUGC	208
692	692-710	GCGGUUCAGCAACAACCCUGCCC	209
698	698-716	CAGCAACAACCCUGCCCUGUGCA	210
700	700-718	GCAACAACCCUGCCCUGUGCAAC	211
719	719-737	CAACGUGGAGAGCAUCCAGUGGC	212
720	720-738	AACGUGGAGAGCAUCCAGUGGCG	213
721	721-739	ACGUGGAGAGCAUCCAGUGGCGG	214
724	724-742	UGGAGAGCAUCCAGUGGCGGGAC	215
725	725-743	GGAGAGCAUCCAGUGGCGGGACA	216
726	726-744	GAGAGCAUCCAGUGGCGGGACAU	217
733	733-751	UCCAGUGGCGGGACAUAGUCAGC	218
734	734-752	CCAGUGGCGGGACAUAGUCAGCA	219
736	736-754	AGUGGCGGGACAUAGUCAGCAGU	220
737	737-755	GUGGCGGGACAUAGUCAGCAGUG	221
763	763-781	UUCUCAGCAACAUGUCGAUGGAC	222

765	765-783	CUCAGCAACAUGUCGAUGGACUU	223
766	766-784	UCAGCAACAUGUCGAUGGACUUC	224
767	767-785	CAGCAACAUGUCGAUGGACUUCC	225
769	769-787	GCAACAUGUCGAUGGACUUCCAG	226
770	770-788	CAACAUGUCGAUGGACUUCCAGA	227
771	771-789	AACAUGUCGAUGGACUUCCAGAA	228
772	772-790	ACAUGUCGAUGGACUUCCAGAAC	229
775	775-793	UGUCGAUGGACUUCCAGAACCAC	230
789	789-807	CAGAACCACCUGGGCAGCUGCCA	231
798	798-816	CUGGGCAGCUGCCAAAAGUGUGA	232
800	800-818	GGGCAGCUGCCAAAAGUGUGAUC	233
805	805-823	GCUGCCAAAAGUGUGAUCCAAGC	234
806	806-824	CUGCCAAAAGUGUGAUCCAAGCU	235
807	807-825	UGCCAAAAGUGUGAUCCAAGCUG	236
810	810-828	CAAAAGUGUGAUCCAAGCUGUCC	237
814	814-832	AGUGUGAUCCAAGCUGUCCCAAU	238
815	815-833	GUGUGAUCCAAGCUGUCCCAAUG	239
817	817-835	GUGAUCCAAGCUGUCCCAAUGGG	240
818	818-836	UGAUCCAAGCUGUCCCAAUGGGA	241
819	819-837	GAUCCAAGCUGUCCCAAUGGGAG	242
820	820-838	AUCCAAGCUGUCCCAAUGGGAGC	243
821	821-839	UCCAAGCUGUCCCAAUGGGAGCU	244
823	823-841	CAAGCUGUCCCAAUGGGAGCUGC	245
826	826-844	GCUGUCCCAAUGGGAGCUGCUGG	246
847	847-865	GGGGUGCAGGAGAGGAGAACUGC	247
871	871-889	AGAAACUGACCAAAAUCAUCUGU	248
872	872-890	GAAACUGACCAAAAUCAUCUGUG	249
873	873-891	AAACUGACCAAAAUCAUCUGUGC	250
877	877-895	UGACCAAAAUCAUCUGUGCCCAG	251
878	878-896	GACCAAAAUCAUCUGUGCCCAGC	252
881	881-899	CAAAAUCAUCUGUGCCCAGCAGU	253
890	890-908	CUGUGCCCAGCAGUGCUCCGGGC	254
892	892-910	GUGCCCAGCAGUGCUCCGGGCGC	255
929	929-947	CCCCAGUGACUGCUGCCACAACC	256
930	930-948	CCCAGUGACUGCUGCCACAACCA	257
979	979-997	GGGAGAGCGACUGCCUGGUCUGC	258
980	980-998	GGAGAGCGACUGCCUGGUCUGCC	259
981	981-999	GAGAGCGACUGCCUGGUCUGCCG	260
982	982-1000	AGAGCGACUGCCUGGUCUGCCGC	261
983	983-1001	GAGCGACUGCCUGGUCUGCCGCA	262
984	984-1002	AGCGACUGCCUGGUCUGCCGCAA	263
989	989-1007	CUGCCUGGUCUGCCGCAAAUUCC	264
990	990-1008	UGCCUGGUCUGCCGCAAAUUCCG	265
991	991-1009	GCCUGGUCUGCCGCAAAUUCCGA	266
992	992-1010	CCUGGUCUGCCGCAAAUUCCGAG	267
994	994-1012	UGGUCUGCCGCAAAUUCCGAGAC	268
995	995-1013	GGUCUGCCGCAAAUUCCGAGACG	269
996	996-1014	GUCUGCCGCAAAUUCCGAGACGA	270
997	997-1015	UCUGCCGCAAAUUCCGAGACGAA	271
999	999-1017	UGCCGCAAAUUCCGAGACGAAGC	272
1004	1004-1022	CAAAUUCCGAGACGAAGCCACGU	273
1005	1005-1023	AAAUUCCGAGACGAAGCCACGUG	274
1006	1006-1024	AAUUCCGAGACGAAGCCACGUGC	275

1007	1007-1025	AUUCCGAGACGAAGCCACGUGCA	276
1008	1008-1026	UUCCGAGACGAAGCCACGUGCAA	277
1010	1010-1028	CCGAGACGAAGCCACGUGCAAGG	278
1013	1013-1031	AGACGAAGCCACGUGCAAGGACA	279
1014	1014-1032	GACGAAGCCACGUGCAAGGACAC	280
1015	1015-1033	ACGAAGCCACGUGCAAGGACACC	281
1016	1016-1034	CGAAGCCACGUGCAAGGACACCU	282
1040	1040-1058	CCCCCCACUCAUGCUCUACAACC	283
1042	1042-1060	CCCCACUCAUGCUCUACAACCCC	284
1044	1044-1062	CCACUCAUGCUCUACAACCCCAC	285
1047	1047-1065	CUCAUGCUCUACAACCCCACCAC	286
1071	1071-1089	UACCAGAUGGAUGUGAACCCCGA	287
1073	1073-1091	CCAGAUGGAUGUGAACCCCGAGG	288
1074	1074-1092	CAGAUGGAUGUGAACCCCGAGGG	289
1075	1075-1093	AGAUGGAUGUGAACCCCGAGGGC	290
1077	1077-1095	AUGGAUGUGAACCCCGAGGGCAA	291
1078	1078-1096	UGGAUGUGAACCCCGAGGGCAAA	292
1080	1080-1098	GAUGUGAACCCCGAGGGCAAAUA	293
1084	1084-1102	UGAACCCCGAGGGCAAAUACAGC	294
1085	1085-1103	GAACCCCGAGGGCAAAUACAGCU	295
1087	1087-1105	ACCCCGAGGGCAAAUACAGCUUU	296
1088	1088-1106	CCCCGAGGGCAAAUACAGCUUUG	297
1089	1089-1107	CCCGAGGGCAAAUACAGCUUUGG	298
1096	1096-1114	GCAAAUACAGCUUUGGUGCCACC	299
1097	1097-1115	CAAAUACAGCUUUGGUGCCACCU	300
1098	1098-1116	AAAUACAGCUUUGGUGCCACCUG	301
1104	1104-1122	AGCUUUGGUGCCACCUGCGUGAA	302
1106	1106-1124	CUUUGGUGCCACCUGCGUGAAGA	303
1112	1112-1130	UGCCACCUGCGUGAAGAAGUGUC	304
1116	1116-1134	ACCUGCGUGAAGAAGUGUCCCCG	305
1117	1117-1135	CCUGCGUGAAGAAGUGUCCCCGU	306
1118	1118-1136	CUGCGUGAAGAAGUGUCCCCGUA	307
1119	1119-1137	UGCGUGAAGAAGUGUCCCCGUAA	308
1120	1120-1138	GCGUGAAGAAGUGUCCCCGUAAU	309
1121	1121-1139	CGUGAAGAAGUGUCCCCGUAAUU	310
1122	1122-1140	GUGAAGAAGUGUCCCCGUAAUUA	311
1123	1123-1141	UGAAGAAGUGUCCCCGUAAUUAU	312
1124	1124-1142	GAAGAAGUGUCCCCGUAAUUAUG	313
1125	1125-1143	AAGAAGUGUCCCCGUAAUUAUGU	314
1126	1126-1144	AGAAGUGUCCCCGUAAUUAUGUG	315
1127	1127-1145	GAAGUGUCCCCGUAAUUAUGUGG	316
1128	1128-1146	AAGUGUCCCCGUAAUUAUGUGGU	317
1129	1129-1147	AGUGUCCCCGUAAUUAUGUGGUG	318
1130	1130-1148	GUGUCCCCGUAAUUAUGUGGUGA	319
1132	1132-1150	GUCCCCGUAAUUAUGUGGUGACA	320
1134	1134-1152	CCCCGUAAUUAUGUGGUGACAGA	321
1136	1136-1154	CCGUAAUUAUGUGGUGACAGAUC	322
1137	1137-1155	CGUAAUUAUGUGGUGACAGAUCA	323
1138	1138-1156	GUAAUUAUGUGGUGACAGAUCAC	324
1139	1139-1157	UAAUUAUGUGGUGACAGAUCACG	325
1140	1140-1158	AAUUAUGUGGUGACAGAUCACGG	326
1142	1142-1160	UUAUGUGGUGACAGAUCACGGCU	327
1145	1145-1163	UGUGGUGACAGAUCACGGCUCGU	328

1147	1147-1165	UGGUGACAGAUCACGGCUCGUGC	329
1148	1148-1166	GGUGACAGAUCACGGCUCGUGCG	330
1149	1149-1167	GUGACAGAUCACGGCUCGUGCGU	331
1150	1150-1168	UGACAGAUCACGGCUCGUGCGUC	332
1151	1151-1169	GACAGAUCACGGCUCGUGCGUCC	333
1152	1152-1170	ACAGAUCACGGCUCGUGCGUCCG	334
1153	1153-1171	CAGAUCACGGCUCGUGCGUCCGA	335
1154	1154-1172	AGAUCACGGCUCGUGCGUCCGAG	336
1155	1155-1173	GAUCACGGCUCGUGCGUCCGAGC	337
1156	1156-1174	AUCACGGCUCGUGCGUCCGAGCC	338
1157	1157-1175	UCACGGCUCGUGCGUCCGAGCCU	339
1160	1160-1178	CGGCUCGUGCGUCCGAGCCUGUG	340
1200	1200-1218	AUGGAGGAAGACGGCGUCCGCAA	341
1201	1201-1219	UGGAGGAAGACGGCGUCCGCAAG	342
1203	1203-1221	GAGGAAGACGGCGUCCGCAAGUG	343
1204	1204-1222	AGGAAGACGGCGUCCGCAAGUGU	344
1205	1205-1223	GGAAGACGGCGUCCGCAAGUGUA	345
1207	1207-1225	AAGACGGCGUCCGCAAGUGUAAG	346
1208	1208-1226	AGACGGCGUCCGCAAGUGUAAGA	347
1211	1211-1229	CGGCGUCCGCAAGUGUAAGAAGU	348
1212	1212-1230	GGCGUCCGCAAGUGUAAGAAGUG	349
1213	1213-1231	GCGUCCGCAAGUGUAAGAAGUGC	350
1214	1214-1232	CGUCCGCAAGUGUAAGAAGUGCG	351
1215	1215-1233	GUCCGCAAGUGUAAGAAGUGCGA	352
1216	1216-1234	UCCGCAAGUGUAAGAAGUGCGAA	353
1217	1217-1235	CCGCAAGUGUAAGAAGUGCGAAG	354
1219	1219-1237	GCAAGUGUAAGAAGUGCGAAGGG	355
1220	1220-1238	CAAGUGUAAGAAGUGCGAAGGGC	356
1221	1221-1239	AAGUGUAAGAAGUGCGAAGGGCC	357
1222	1222-1240	AGUGUAAGAAGUGCGAAGGGCCU	358
1223	1223-1241	GUGUAAGAAGUGCGAAGGGCCUU	359
1224	1224-1242	UGUAAGAAGUGCGAAGGGCCUUG	360
1225	1225-1243	GUAAGAAGUGCGAAGGGCCUUGC	361
1226	1226-1244	UAAGAAGUGCGAAGGGCCUUGCC	362
1229	1229-1247	GAAGUGCGAAGGGCCUUGCCGCA	363
1230	1230-1248	AAGUGCGAAGGGCCUUGCCGCAA	364
1231	1231-1249	AGUGCGAAGGGCCUUGCCGCAAA	365
1232	1232-1250	GUGCGAAGGGCCUUGCCGCAAAG	366
1233	1233-1251	UGCGAAGGGCCUUGCCGCAAAGU	367
1235	1235-1253	CGAAGGGCCUUGCCGCAAAGUGU	368
1236	1236-1254	GAAGGGCCUUGCCGCAAAGUGUG	369
1237	1237-1255	AAGGGCCUUGCCGCAAAGUGUGU	370
1238	1238-1256	AGGGCCUUGCCGCAAAGUGUGUA	371
1239	1239-1257	GGGCCUUGCCGCAAAGUGUGUAA	372
1241	1241-1259	GCCUUGCCGCAAAGUGUGUAACG	373
1261	1261-1279	ACGGAAUAGGUAUUGGUGAAUUU	374
1262	1262-1280	CGGAAUAGGUAUUGGUGAAUUUA	375
1263	1263-1281	GGAAUAGGUAUUGGUGAAUUUAA	376
1264	1264-1282	GAAUAGGUAUUGGUGAAUUUAAA	377
1266	1266-1284	AUAGGUAUUGGUGAAUUUAAAGA	378
1267	1267-1285	UAGGUAUUGGUGAAUUUAAAGAC	379
1289	1289-1307	CUCACUCUCCAUAAAUGCUACGA	380
1313	1313-1331	UAUUAAACACUUCAAAAACUGCA	381

1320	1320-1338	CACUUCAAAAACUGCACCUCCAU	382
1321	1321-1339	ACUUCAAAAACUGCACCUCCAUC	383
1322	1322-1340	CUUCAAAAACUGCACCUCCAUCA	384
1323	1323-1341	UUCAAAAACUGCACCUCCAUCAG	385
1324	1324-1342	UCAAAAACUGCACCUCCAUCAGU	386
1328	1328-1346	AAACUGCACCUCCAUCAGUGGCG	387
1332	1332-1350	UGCACCUCCAUCAGUGGCGAUCU	388
1333	1333-1351	GCACCUCCAUCAGUGGCGAUCUC	389
1335	1335-1353	ACCUCCAUCAGUGGCGAUCUCCA	390
1338	1338-1356	UCCAUCAGUGGCGAUCUCCACAU	391
1344	1344-1362	AGUGGCGAUCUCCACAUCCUGCC	392
1345	1345-1363	GUGGCGAUCUCCACAUCCUGCCG	393
1346	1346-1364	UGGCGAUCUCCACAUCCUGCCGG	394
1347	1347-1365	GGCGAUCUCCACAUCCUGCCGGU	395
1348	1348-1366	GCGAUCUCCACAUCCUGCCGGUG	396
1353	1353-1371	CUCCACAUCCUGCCGGUGGCAUU	397
1354	1354-1372	UCCACAUCCUGCCGGUGGCAUUU	398
1355	1355-1373	CCACAUCCUGCCGGUGGCAUUUA	399
1357	1357-1375	ACAUCCUGCCGGUGGCAUUUAGG	400
1360	1360-1378	UCCUGCCGGUGGCAUUUAGGGGU	401
1361	1361-1379	CCUGCCGGUGGCAUUUAGGGGUG	402
1362	1362-1380	CUGCCGGUGGCAUUUAGGGGUGA	403
1363	1363-1381	UGCCGGUGGCAUUUAGGGGUGAC	404
1366	1366-1384	CGGUGGCAUUUAGGGGUGACUCC	405
1369	1369-1387	UGGCAUUUAGGGGUGACUCCUUC	406
1370	1370-1388	GGCAUUUAGGGGUGACUCCUUCA	407
1371	1371-1389	GCAUUUAGGGGUGACUCCUUCAC	408
1372	1372-1390	CAUUUAGGGGUGACUCCUUCACA	409
1373	1373-1391	AUUUAGGGGUGACUCCUUCACAC	410
1374	1374-1392	UUUAGGGGUGACUCCUUCACACA	411
1404	1404-1422	CCUCUGGAUCCACAGGAACUGGA	412
1408	1408-1426	UGGAUCCACAGGAACUGGAUAUU	413
1409	1409-1427	GGAUCCACAGGAACUGGAUAUUC	414
1411	1411-1429	AUCCACAGGAACUGGAUAUUCUG	415
1412	1412-1430	UCCACAGGAACUGGAUAUUCUGA	416
1419	1419-1437	GAACUGGAUAUUCUGAAAACCGU	417
1426	1426-1444	AUAUUCUGAAAACCGUAAAGGAA	418
1427	1427-1445	UAUUCUGAAAACCGUAAAGGAAA	419
1430	1430-1448	UCUGAAAACCGUAAAGGAAAUCA	420
1431	1431-1449	CUGAAAACCGUAAAGGAAAUCAC	421

Table 4. EGFR siRNA Sequences

hs Id #	**Sequence position in NM_005228.3**	**sense strand sequence (5′-3′)**	**SEQ ID NO:**	**antisense strand sequence (5′-3′)**	**SEQ ID NO:**
68	68-86	CGGCCGGAGUCCCGAGCUATT	422	UAGCUCGGGACUCCGGCCGTT	423
71	71-89	CCGGAGUCCCGAGCUAGCCTT	424	GGCUAGCUCGGGACUCCGGTT	425
72	72-90	CGGAGUCCCGAGCUAGCCCTT	426	GGGCUAGCUCGGGACUCCGTT	427

73	73-91	GGAGUCCCGAGCUAGCCC CTT	428	GGGGCUAGCUCGGGACUC CTT	429
74	74-92	GAGUCCCGAGCUAGCCCC GTT	430	CGGGGCUAGCUCGGGACU CTT	431
75	75-93	AGUCCCGAGCUAGCCCCG GTT	432	CCGGGGCUAGCUCGGGAC UTT	433
76	76-94	GUCCCGAGCUAGCCCCGG CTT	434	GCCGGGGCUAGCUCGGGA CTT	435
78	78-96	CCCGAGCUAGCCCCGGCG GTT	436	CCGCCGGGGCUAGCUCGG GTT	437
114	114-132	GGACGACAGGCCACCUCG UTT	438	ACGAGGUGGCCUGUCGUC CTT	439
115	115-133	GACGACAGGCCACCUCGU CTT	440	GACGAGGUGGCCUGUCGU CTT	441
116	116-134	ACGACAGGCCACCUCGUC GTT	442	CGACGAGGUGGCCUGUCG UTT	443
117	117-135	CGACAGGCCACCUCGUCG GTT	444	CCGACGAGGUGGCCUGUC GTT	445
118	118-136	GACAGGCCACCUCGUCGG CTT	446	GCCGACGAGGUGGCCUGU CTT	447
120	120-138	CAGGCCACCUCGUCGGCG UTT	448	ACGCCGACGAGGUGGCCU GTT	449
121	121-139	AGGCCACCUCGUCGGCGU CTT	450	GACGCCGACGAGGUGGCC UTT	451
122	122-140	GGCCACCUCGUCGGCGUC CTT	452	GGACGCCGACGAGGUGGC CTT	453
123	123-141	GCCACCUCGUCGGCGUCC GTT	454	CGGACGCCGACGAGGUGG CTT	455
124	124-142	CCACCUCGUCGGCGUCCG CTT	456	GCGGACGCCGACGAGGUG GTT	457
125	125-143	CACCUCGUCGGCGUCCGC CTT	458	GGCGGACGCCGACGAGGU GTT	459
126	126-144	ACCUCGUCGGCGUCCGCC CTT	460	GGGCGGACGCCGACGAGG UTT	461
127	127-145	CCUCGUCGGCGUCCGCCC GTT	462	CGGGCGGACGCCGACGAG GTT	463
128	128-146	CUCGUCGGCGUCCGCCCG ATT	464	UCGGGCGGACGCCGACGA GTT	465
129	129-147	UCGUCGGCGUCCGCCCGA GTT	466	CUCGGGCGGACGCCGACG ATT	467
130	130-148	CGUCGGCGUCCGCCCGAG UTT	468	ACUCGGGCGGACGCCGAC GTT	469
131	131-149	GUCGGCGUCCGCCCGAGU CTT	470	GACUCGGGCGGACGCCGA CTT	471
132	132-150	UCGGCGUCCGCCCGAGUC CTT	472	GGACUCGGGCGGACGCCG ATT	473
135	135-153	GCGUCCGCCCGAGUCCCC GTT	474	CGGGGACUCGGGCGGACG CTT	475
136	136-154	CGUCCGCCCGAGUCCCCG CTT	476	GCGGGGACUCGGGCGGAC GTT	477
141	141-159	GCCCGAGUCCCCGCCUCG CTT	478	GCGAGGCGGGGACUCGGG CTT	479
164	164-182	AACGCCACAACCACCGCG CTT	480	GCGCGGUGGUUGUGGCGU UTT	481
165	165-183	ACGCCACAACCACCGCGC	482	UGCGCGGUGGUUGUGGCG	483

		ATT		UTT	
166	166-184	CGCCACAACCACCGCGCA CTT	484	GUGCGCGGUGGUUGUGGC GTT	485
168	168-186	CCACAACCACCGCGCACG GTT	486	CCGUGCGCGGUGGUUGUG GTT	487
169	169-187	CACAACCACCGCGCACGG CTT	488	GCCGUGCGCGGUGGUUGU GTT	489
170	170-188	ACAACCACCGCGCACGGC CTT	490	GGCCGUGCGCGGUGGUUG UTT	491
247	247-265	AUGCGACCCUCCGGGACG GTT	492	CCGUCCCGGAGGGUCGCA UTT	493
248	248-266	UGCGACCCUCCGGGACGG CTT	494	GCCGUCCCGGAGGGUCGC ATT	495
249	249-267	GCGACCCUCCGGGACGGC CTT	496	GGCCGUCCCGGAGGGUCG CTT	497
251	251-269	GACCCUCCGGGACGGCCG GTT	498	CCGGCCGUCCCGGAGGGU CTT	499
252	252-270	ACCCUCCGGGACGGCCGG GTT	500	CCCGGCCGUCCCGGAGGG UTT	501
254	254-272	CCUCCGGGACGGCCGGGG CTT	502	GCCCCGGCCGUCCCGGAG GTT	503
329	329-347	AGAAAGUUUGCCAAGGCA CTT	504	GUGCCUUGGCAAACUUUC UTT	505
330	330-348	GAAAGUUUGCCAAGGCAC GTT	506	CGUGCCUUGGCAAACUUU CTT	507
332	332-350	AAGUUUGCCAAGGCACGA GTT	508	CUCGUGCCUUGGCAAACU UTT	509
333	333-351	AGUUUGCCAAGGCACGAG UTT	510	ACUCGUGCCUUGGCAAAC UTT	511
334	334-352	GUUUGCCAAGGCACGAGU ATT	512	UACUCGUGCCUUGGCAAA CTT	513
335	335-353	UUUGCCAAGGCACGAGUA ATT	514	UUACUCGUGCCUUGGCAA ATT	515
336	336-354	UUGCCAAGGCACGAGUAA CTT	516	GUUACUCGUGCCUUGGCA ATT	517
337	337-355	UGCCAAGGCACGAGUAAC ATT	518	UGUUACUCGUGCCUUGGC ATT	519
338	338-356	GCCAAGGCACGAGUAACA ATT	520	UUGUUACUCGUGCCUUGG CTT	521
361	361-379	ACGCAGUUGGGCACUUUU GTT	522	CAAAAGUGCCCAACUGCG UTT	523
362	362-380	CGCAGUUGGGCACUUUUG ATT	524	UCAAAAGUGCCCAACUGC GTT	525
363	363-381	GCAGUUGGGCACUUUUGA ATT	526	UUCAAAAGUGCCCAACUG CTT	527
364	364-382	CAGUUGGGCACUUUUGAA GTT	528	CUUCAAAAGUGCCCAACU GTT	529
365	365-383	AGUUGGGCACUUUUGAAG ATT	530	UCUUCAAAAGUGCCCAAC UTT	531
366	366-384	GUUGGGCACUUUUGAAGA UTT	532	AUCUUCAAAAGUGCCCAA CTT	533
367	367-385	UUGGGCACUUUUGAAGAU CTT	534	GAUCUUCAAAAGUGCCCA ATT	535
368	368-386	UGGGCACUUUUGAAGAUC ATT	536	UGAUCUUCAAAAGUGCCC ATT	537

369	369-387	GGGCACUUUUGAAGAUCAUTT	538	AUGAUCUUCAAAAGUGCCCTT	539
377	377-395	UUGAAGAUCAUUUUCUCAGTT	540	CUGAGAAAAUGAUCUUCAATT	541
379	379-397	GAAGAUCAUUUUCUCAGCCTT	542	GGCUGAGAAAAUGAUCUUCTT	543
380	380-398	AAGAUCAUUUUCUCAGCCUTT	544	AGGCUGAGAAAAUGAUCUUTT	545
385	385-403	CAUUUUCUCAGCCUCCAGATT	546	UCUGGAGGCUGAGAAAAUGTT	547
394	394-412	AGCCUCCAGAGGAUGUUCATT	548	UGAACAUCCUCUGGAGGCUTT	549
396	396-414	CCUCCAGAGGAUGUUCAAUTT	550	AUUGAACAUCCUCUGGAGGTT	551
397	397-415	CUCCAGAGGAUGUUCAAUATT	552	UAUUGAACAUCCUCUGGAGTT	553
401	401-419	AGAGGAUGUUCAAUAACUGTT	554	CAGUUAUUGAACAUCCUCUTT	555
403	403-421	AGGAUGUUCAAUAACUGUGTT	556	CACAGUUAUUGAACAUCCUTT	557
407	407-425	UGUUCAAUAACUGUGAGGUTT	558	ACCUCACAGUUAUUGAACATT	559
409	409-427	UUCAAUAACUGUGAGGUGGTT	560	CCACCUCACAGUUAUUGAATT	561
410	410-428	UCAAUAACUGUGAGGUGGUTT	562	ACCACCUCACAGUUAUUGATT	563
411	411-429	CAAUAACUGUGAGGUGGUCTT	564	GACCACCUCACAGUUAUUGTT	565
412	412-430	AAUAACUGUGAGGUGGUCCTT	566	GGACCACCUCACAGUUAUUTT	567
413	413-431	AUAACUGUGAGGUGGUCCUTT	568	AGGACCACCUCACAGUUATT	569
414	414-432	UAACUGUGAGGUGGUCCUUTT	570	AAGGACCACCUCACAGUUATT	571
416	416-434	ACUGUGAGGUGGUCCUUGGTT	572	CCAAGGACCACCUCACAGUTT	573
418	418-436	UGUGAGGUGGUCCUUGGGATT	574	UCCCAAGGACCACCUCACATT	575
419	419-437	GUGAGGUGGUCCUUGGGAATT	576	UUCCCAAGGACCACCUCACTT	577
425	425-443	UGGUCCUUGGGAAUUUGGATT	578	UCCAAAUUCCCAAGGACCATT	579
431	431-449	UUGGGAAUUUGGAAAUUACTT	580	GUAAUUUCCAAAUUCCCAATT	581
432	432-450	UGGGAAUUUGGAAAUUACCTT	582	GGUAAUUUCCAAAUUCCCATT	583
433	433-451	GGGAAUUUGGAAAUUACCUTT	584	AGGUAAUUUCCAAAUUCCCTT	585
434	434-452	GGAAUUUGGAAAUUACCUATT	586	UAGGUAAUUUCCAAAUUCCTT	587
458	458-476	AGAGGAAUUAUGAUCUUUCTT	588	GAAAGAUCAUAAUUCCUCUTT	589
459	459-477	GAGGAAUUAUGAUCUUUCCTT	590	GGAAAGAUCAUAAUUCCUCTT	591
463	463-481	AAUUAUGAUCUUUCCUUC	592	AGAAGGAAAGAUCAUAAU	593

		UTT		UTT	
464	464-482	AUUAUGAUCUUUCCUUCU UTT	594	AAGAAGGAAAGAUCAUAA UTT	595
466	466-484	UAUGAUCUUUCCUUCUUA ATT	596	UUAAGAAGGAAAGAUCAU ATT	597
468	468-486	UGAUCUUUCCUUCUUAAA GTT	598	CUUUAAGAAGGAAAGAUC ATT	599
471	471-489	UCUUUCCUUCUUAAAGAC CTT	600	GGUCUUUAAGAAGGAAAG ATT	601
476	476-494	CCUUCUUAAAGACCAUCC ATT	602	UGGAUGGUCUUUAAGAAG GTT	603
477	477-495	CUUCUUAAAGACCAUCCA GTT	604	CUGGAUGGUCUUUAAGAA GTT	605
479	479-497	UCUUAAAGACCAUCCAGG ATT	606	UCCUGGAUGGUCUUUAAG ATT	607
481	481-499	UUAAAGACCAUCCAGGAG GTT	608	CCUCCUGGAUGGUCUUUA ATT	609
482	482-500	UAAAGACCAUCCAGGAGG UTT	610	ACCUCCUGGAUGGUCUUU ATT	611
492	492-510	CCAGGAGGUGGCUGGUUA UTT	612	AUAACCAGCCACCUCCUG GTT	613
493	493-511	CAGGAGGUGGCUGGUUAU GTT	614	CAUAACCAGCCACCUCCU GTT	615
494	494-512	AGGAGGUGGCUGGUUAUG UTT	616	ACAUAACCAGCCACCUCC UTT	617
495	495-513	GGAGGUGGCUGGUUAUGU CTT	618	GACAUAACCAGCCACCUC CTT	619
496	496-514	GAGGUGGCUGGUUAUGUC CTT	620	GGACAUAACCAGCCACCU CTT	621
497	497-515	AGGUGGCUGGUUAUGUCC UTT	622	AGGACAUAACCAGCCACC UTT	623
499	499-517	GUGGCUGGUUAUGUCCUC ATT	624	UGAGGACAUAACCAGCCA CTT	625
520	520-538	GCCCUCAACACAGUGGAG CTT	626	GCUCCACUGUGUUGAGGG CTT	627
542	542-560	UUCCUUUGGAAAACCUGC ATT	628	UGCAGGUUUUCCAAAGGA ATT	629
543	543-561	UCCUUUGGAAAACCUGCA GTT	630	CUGCAGGUUUUCCAAAGG ATT	631
550	550-568	GAAAACCUGCAGAUCAUC ATT	632	UGAUGAUCUGCAGGUUUU CTT	633
551	551-569	AAAACCUGCAGAUCAUCA GTT	634	CUGAUGAUCUGCAGGUUU UTT	635
553	553-571	AACCUGCAGAUCAUCAGA GTT	636	CUCUGAUGAUCUGCAGGU UTT	637
556	556-574	CUGCAGAUCAUCAGAGGA ATT	638	UUCCUCUGAUGAUCUGCA GTT	639
586	586-604	GAAAAUUCCUAUGCCUUA GTT	640	CUAAGGCAUAGGAAUUUU CTT	641
587	587-605	AAAAUUCCUAUGCCUUAG CTT	642	GCUAAGGCAUAGGAAUUU UTT	643
589	589-607	AAUUCCUAUGCCUUAGCA GTT	644	CUGCUAAGGCAUAGGAAU UTT	645
592	592-610	UCCUAUGCCUUAGCAGUC UTT	646	AGACUGCUAAGGCAUAGG ATT	647

593	593-611	CCUAUGCCUUAGCAGUCU UTT	648	AAGACUGCUAAGGCAUAG GTT	649
594	594-612	CUAUGCCUUAGCAGUCUU ATT	650	UAAGACUGCUAAGGCAUA GTT	651
596	596-614	AUGCCUUAGCAGUCUUAU CTT	652	GAUAAGACUGCUAAGGCA UTT	653
597	597-615	UGCCUUAGCAGUCUUAUC UTT	654	AGAUAAGACUGCUAAGGC ATT	655
598	598-616	GCCUUAGCAGUCUUAUCU ATT	656	UAGAUAAGACUGCUAAGG CTT	657
599	599-617	CCUUAGCAGUCUUAUCUA ATT	658	UUAGAUAAGACUGCUAAG GTT	659
600	600-618	CUUAGCAGUCUUAUCUAA CTT	660	GUUAGAUAAGACUGCUAA GTT	661
601	601-619	UUAGCAGUCUUAUCUAAC UTT	662	AGUUAGAUAAGACUGCUA ATT	663
602	602-620	UAGCAGUCUUAUCUAACU ATT	664	UAGUUAGAUAAGACUGCU ATT	665
603	603-621	AGCAGUCUUAUCUAACUA UTT	666	AUAGUUAGAUAAGACUGC UTT	667
604	604-622	GCAGUCUUAUCUAACUAU GTT	668	CAUAGUUAGAUAAGACUG CTT	669
605	605-623	CAGUCUUAUCUAACUAUG ATT	670	UCAUAGUUAGAUAAGACU GTT	671
608	608-626	UCUUAUCUAACUAUGAUG CTT	672	GCAUCAUAGUUAGAUAAG ATT	673
609	609-627	CUUAUCUAACUAUGAUGC ATT	674	UGCAUCAUAGUUAGAUAA GTT	675
610	610-628	UUAUCUAACUAUGAUGCA ATT	676	UUGCAUCAUAGUUAGAUA ATT	677
611	611-629	UAUCUAACUAUGAUGCAA ATT	678	UUUGCAUCAUAGUUAGAU ATT	679
612	612-630	AUCUAACUAUGAUGCAAA UTT	680	AUUUGCAUCAUAGUUAGA UTT	681
613	613-631	UCUAACUAUGAUGCAAAU ATT	682	UAUUUGCAUCAUAGUUAG ATT	683
614	614-632	CUAACUAUGAUGCAAAUA ATT	684	UUAUUUGCAUCAUAGUUA GTT	685
616	616-634	AACUAUGAUGCAAAUAAA ATT	686	UUUUAUUUGCAUCAUAGU UTT	687
622	622-640	GAUGCAAAUAAAACCGGA CTT	688	GUCCGGUUUUAUUUGCAU CTT	689
623	623-641	AUGCAAAUAAAACCGGAC UTT	690	AGUCCGGUUUUAUUUGCA UTT	691
624	624-642	UGCAAAUAAAACCGGACU GTT	692	CAGUCCGGUUUUAUUUGC ATT	693
626	626-644	CAAAUAAAACCGGACUGA ATT	694	UUCAGUCCGGUUUUAUUU GTT	695
627	627-645	AAAUAAAACCGGACUGAA GTT	696	CUUCAGUCCGGUUUUAUU UTT	697
628	628-646	AAUAAAACCGGACUGAAG GTT	698	CCUUCAGUCCGGUUUUAU UTT	699
630	630-648	UAAAACCGGACUGAAGGA GTT	700	CUCCUUCAGUCCGGUUUU ATT	701
631	631-649	AAAACCGGACUGAAGGAG	702	GCUCCUUCAGUCCGGUUU	703

		CTT		UTT	
632	632-650	AAACCGGACUGAAGGAGC UTT	704	AGCUCCUUCAGUCCGGUU UTT	705
633	633-651	AACCGGACUGAAGGAGCU GTT	706	CAGCUCCUUCAGUCCGGU UTT	707
644	644-662	AGGAGCUGCCCAUGAGAA ATT	708	UUUCUCAUGGGCAGCUCC UTT	709
665	665-683	UACAGGAAAUCCUGCAUG GTT	710	CCAUGCAGGAUUUCCUGU ATT	711
668	668-686	AGGAAAUCCUGCAUGGCG CTT	712	GCGCCAUGCAGGAUUUCC UTT	713
669	669-687	GGAAAUCCUGCAUGGCGC CTT	714	GGCGCCAUGCAGGAUUUC CTT	715
670	670-688	GAAAUCCUGCAUGGCGCC GTT	716	CGGCGCCAUGCAGGAUUU CTT	717
671	671-689	AAAUCCUGCAUGGCGCCG UTT	718	ACGGCGCCAUGCAGGAUU UTT	719
672	672-690	AAUCCUGCAUGGCGCCGU GTT	720	CACGGCGCCAUGCAGGAU UTT	721
674	674-692	UCCUGCAUGGCGCCGUGC GTT	722	CGCACGGCGCCAUGCAGG ATT	723
676	676-694	CUGCAUGGCGCCGUGCGG UTT	724	ACCGCACGGCGCCAUGCA GTT	725
677	677-695	UGCAUGGCGCCGUGCGGU UTT	726	AACCGCACGGCGCCAUGC ATT	727
678	678-696	GCAUGGCGCCGUGCGGUU CTT	728	GAACCGCACGGCGCCAUG CTT	729
680	680-698	AUGGCGCCGUGCGGUUCA GTT	730	CUGAACCGCACGGCGCCA UTT	731
681	681-699	UGGCGCCGUGCGGUUCAG CTT	732	GCUGAACCGCACGGCGCC ATT	733
682	682-700	GGCGCCGUGCGGUUCAGC ATT	734	UGCUGAACCGCACGGCGC CTT	735
683	683-701	GCGCCGUGCGGUUCAGCA ATT	736	UUGCUGAACCGCACGGCG CTT	737
684	684-702	CGCCGUGCGGUUCAGCAA CTT	738	GUUGCUGAACCGCACGGC GTT	739
685	685-703	GCCGUGCGGUUCAGCAAC ATT	740	UGUUGCUGAACCGCACGG CTT	741
686	686-704	CCGUGCGGUUCAGCAACA ATT	742	UUGUUGCUGAACCGCACG GTT	743
688	688-706	GUGCGGUUCAGCAACAAC CTT	744	GGUUGUUGCUGAACCGCA CTT	745
690	690-708	GCGGUUCAGCAACAACCC UTT	746	AGGGUUGUUGCUGAACCG CTT	747
692	692-710	GGUUCAGCAACAACCCUG CTT	748	GCAGGGUUGUUGCUGAAC CTT	749
698	698-716	GCAACAACCCUGCCCUGU GTT	750	CACAGGGCAGGGUUGUUG CTT	751
700	700-718	AACAACCCUGCCCUGUGC ATT	752	UGCACAGGGCAGGGUUGU UTT	753
719	719-737	ACGUGGAGAGCAUCCAGU GTT	754	CACUGGAUGCUCUCCACG UTT	755
720	720-738	CGUGGAGAGCAUCCAGUG GTT	756	CCACUGGAUGCUCUCCAC GTT	757

721	721-739	GUGGAGAGCAUCCAGUGG CTT	758	GCCACUGGAUGCUCUCCA CTT	759
724	724-742	GAGAGCAUCCAGUGGCGG GTT	760	CCCGCCACUGGAUGCUCU CTT	761
725	725-743	AGAGCAUCCAGUGGCGGG ATT	762	UCCCGCCACUGGAUGCUC UTT	763
726	726-744	GAGCAUCCAGUGGCGGGA CTT	764	GUCCCGCCACUGGAUGCU CTT	765
733	733-751	CAGUGGCGGGACAUAGUC ATT	766	UGACUAUGUCCCGCCACU GTT	767
734	734-752	AGUGGCGGGACAUAGUCA GTT	768	CUGACUAUGUCCCGCCAC UTT	769
736	736-754	UGGCGGGACAUAGUCAGC ATT	770	UGCUGACUAUGUCCCGCC ATT	771
737	737-755	GGCGGGACAUAGUCAGCA GTT	772	CUGCUGACUAUGUCCCGC CTT	773
763	763-781	CUCAGCAACAUGUCGAUG GTT	774	CCAUCGACAUGUUGCUGA GTT	775
765	765-783	CAGCAACAUGUCGAUGGA CTT	776	GUCCAUCGACAUGUUGCU GTT	777
766	766-784	AGCAACAUGUCGAUGGAC UTT	778	AGUCCAUCGACAUGUUGC UTT	779
767	767-785	GCAACAUGUCGAUGGACU UTT	780	AAGUCCAUCGACAUGUUG CTT	781
769	769-787	AACAUGUCGAUGGACUUC CTT	782	GGAAGUCCAUCGACAUGU UTT	783
770	770-788	ACAUGUCGAUGGACUUCC ATT	784	UGGAAGUCCAUCGACAUG UTT	785
771	771-789	CAUGUCGAUGGACUUCCA GTT	786	CUGGAAGUCCAUCGACAU GTT	787
772	772-790	AUGUCGAUGGACUUCCAG ATT	788	UCUGGAAGUCCAUCGACA UTT	789
775	775-793	UCGAUGGACUUCCAGAAC CTT	790	GGUUCUGGAAGUCCAUCG ATT	791
789	789-807	GAACCACCUGGGCAGCUG CTT	792	GCAGCUGCCCAGGUGGUU CTT	793
798	798-816	GGGCAGCUGCCAAAAGUG UTT	794	ACACUUUUGGCAGCUGCC CTT	795
800	800-818	GCAGCUGCCAAAAGUGUG ATT	796	UCACACUUUUGGCAGCUG CTT	797
805	805-823	UGCCAAAAGUGUGAUCCA ATT	798	UUGGAUCACACUUUUGGC ATT	799
806	806-824	GCCAAAAGUGUGAUCCAA GTT	800	CUUGGAUCACACUUUUGG CTT	801
807	807-825	CCAAAAGUGUGAUCCAAG CTT	802	GCUUGGAUCACACUUUUG GTT	803
810	810-828	AAAGUGUGAUCCAAGCUG UTT	804	ACAGCUUGGAUCACACUU UTT	805
814	814-832	UGUGAUCCAAGCUGUCCC ATT	806	UGGGACAGCUUGGAUCAC ATT	807
815	815-833	GUGAUCCAAGCUGUCCCA ATT	808	UUGGGACAGCUUGGAUCA CTT	809
817	817-835	GAUCCAAGCUGUCCCAAU GTT	810	CAUUGGGACAGCUUGGAU CTT	811
818	818-836	AUCCAAGCUGUCCCAAUG	812	CCAUUGGGACAGCUUGGA	813

		GTT		UTT	
819	819-837	UCCAAGCUGUCCCAAUGG GTT	814	CCCAUUGGGACAGCUUGG ATT	815
820	820-838	CCAAGCUGUCCCAAUGGG ATT	816	UCCCAUUGGGACAGCUUG GTT	817
821	821-839	CAAGCUGUCCCAAUGGGA GTT	818	CUCCCAUUGGGACAGCUU GTT	819
823	823-841	AGCUGUCCCAAUGGGAGC UTT	820	AGCUCCCAUUGGGACAGC UTT	821
826	826-844	UGUCCCAAUGGGAGCUGC UTT	822	AGCAGCUCCCAUUGGGAC ATT	823
847	847-865	GGUGCAGGAGAGGAGAAC UTT	824	AGUUCUCCUCUCCUGCAC CTT	825
871	871-889	AAACUGACCAAAAUCAUC UTT	826	AGAUGAUUUUGGUCAGUU UTT	827
872	872-890	AACUGACCAAAAUCAUCU GTT	828	CAGAUGAUUUUGGUCAGU UTT	829
873	873-891	ACUGACCAAAAUCAUCUG UTT	830	ACAGAUGAUUUUGGUCAG UTT	831
877	877-895	ACCAAAAUCAUCUGUGCC CTT	832	GGGCACAGAUGAUUUUGG UTT	833
878	878-896	CCAAAAUCAUCUGUGCCC ATT	834	UGGGCACAGAUGAUUUUG GTT	835
881	881-899	AAAUCAUCUGUGCCCAGC ATT	836	UGCUGGGCACAGAUGAUU UTT	837
890	890-908	GUGCCCAGCAGUGCUCCG GTT	838	CCGGAGCACUGCUGGGCA CTT	839
892	892-910	GCCCAGCAGUGCUCCGGG CTT	840	GCCCGGAGCACUGCUGGG CTT	841
929	929-947	CCAGUGACUGCUGCCACA ATT	842	UUGUGGCAGCAGUCACUG GTT	843
930	930-948	CAGUGACUGCUGCCACAA CTT	844	GUUGUGGCAGCAGUCACU GTT	845
979	979-997	GAGAGCGACUGCCUGGUC UTT	846	AGACCAGGCAGUCGCUCU CTT	847
980	980-998	AGAGCGACUGCCUGGUCU GTT	848	CAGACCAGGCAGUCGCUC UTT	849
981	981-999	GAGCGACUGCCUGGUCUG CTT	850	GCAGACCAGGCAGUCGCU CTT	851
982	982-1000	AGCGACUGCCUGGUCUGC CTT	852	GGCAGACCAGGCAGUCGC UTT	853
983	983-1001	GCGACUGCCUGGUCUGCC GTT	854	CGGCAGACCAGGCAGUCG CTT	855
984	984-1002	CGACUGCCUGGUCUGCCG CTT	856	GCGGCAGACCAGGCAGUC GTT	857
989	989-1007	GCCUGGUCUGCCGCAAAU UTT	858	AAUUUGCGGCAGACCAGG CTT	859
990	990-1008	CCUGGUCUGCCGCAAAUU CTT	860	GAAUUUGCGGCAGACCAG GTT	861
991	991-1009	CUGGUCUGCCGCAAAUUC CTT	862	GGAAUUUGCGGCAGACCA GTT	863
992	992-1010	UGGUCUGCCGCAAAUUCC GTT	864	CGGAAUUUGCGGCAGACC ATT	865
994	994-1012	GUCUGCCGCAAAUUCCGA GTT	866	CUCGGAAUUUGCGGCAGA CTT	867

995	995-1013	UCUGCCGCAAAUUCCGAG ATT	868	UCUCGGAAUUUGCGGCAG ATT	869
996	996-1014	CUGCCGCAAAUUCCGAGA CTT	870	GUCUCGGAAUUUGCGGCA GTT	871
997	997-1015	UGCCGCAAAUUCCGAGAC GTT	872	CGUCUCGGAAUUUGCGGC ATT	873
999	999-1017	CCGCAAAUUCCGAGACGA ATT	874	UUCGUCUCGGAAUUUGCG GTT	875
1004	1004-1022	AAUUCCGAGACGAAGCCA CTT	876	GUGGCUUCGUCUCGGAAU UTT	877
1005	1005-1023	AUUCCGAGACGAAGCCAC GTT	878	CGUGGCUUCGUCUCGGAA UTT	879
1006	1006-1024	UUCCGAGACGAAGCCACG UTT	880	ACGUGGCUUCGUCUCGGA ATT	881
1007	1007-1025	UCCGAGACGAAGCCACGU GTT	882	CACGUGGCUUCGUCUCGG ATT	883
1008	1008-1026	CCGAGACGAAGCCACGUG CTT	884	GCACGUGGCUUCGUCUCG GTT	885
1010	1010-1028	GAGACGAAGCCACGUGCA ATT	886	UUGCACGUGGCUUCGUCU CTT	887
1013	1013-1031	ACGAAGCCACGUGCAAGG ATT	888	UCCUUGCACGUGGCUUCG UTT	889
1014	1014-1032	CGAAGCCACGUGCAAGGA CTT	890	GUCCUUGCACGUGGCUUC GTT	891
1015	1015-1033	GAAGCCACGUGCAAGGAC ATT	892	UGUCCUUGCACGUGGCUU CTT	893
1016	1016-1034	AAGCCACGUGCAAGGACA CTT	894	GUGUCCUUGCACGUGGCU UTT	895
1040	1040-1058	CCCCACUCAUGCUCUACA ATT	896	UUGUAGAGCAUGAGUGGG GTT	897
1042	1042-1060	CCACUCAUGCUCUACAAC CTT	898	GGUUGUAGAGCAUGAGUG GTT	899
1044	1044-1062	ACUCAUGCUCUACAACCC CTT	900	GGGGUUGUAGAGCAUGAG UTT	901
1047	1047-1065	CAUGCUCUACAACCCCAC CTT	902	GGUGGGGUUGUAGAGCAU GTT	903
1071	1071-1089	CCAGAUGGAUGUGAACCC CTT	904	GGGGUUCACAUCCAUCUG GTT	905
1073	1073-1091	AGAUGGAUGUGAACCCCG ATT	906	UCGGGGUUCACAUCCAUC UTT	907
1074	1074-1092	GAUGGAUGUGAACCCCGA GTT	908	CUCGGGGUUCACAUCCAU CTT	909
1075	1075-1093	AUGGAUGUGAACCCCGAG GTT	910	CCUCGGGGUUCACAUCCA UTT	911
1077	1077-1095	GGAUGUGAACCCCGAGGG CTT	912	GCCCUCGGGGUUCACAUC CTT	913
1078	1078-1096	GAUGUGAACCCCGAGGGC ATT	914	UGCCCUCGGGGUUCACAU CTT	915
1080	1080-1098	UGUGAACCCCGAGGGCAA ATT	916	UUUGCCCUCGGGGUUCAC ATT	917
1084	1084-1102	AACCCCGAGGGCAAAUAC ATT	918	UGUAUUUGCCCUCGGGGU UTT	919
1085	1085-1103	ACCCCGAGGGCAAAUACA GTT	920	CUGUAUUUGCCCUCGGGG UTT	921
1087	1087-1105	CCCGAGGGCAAAUACAGC	922	AGCUGUAUUUGCCCUCGG	923

		UTT		GTT	
1088	1088-1106	CCGAGGGCAAAUACAGCU UTT	924	AAGCUGUAUUUGCCCUCG GTT	925
1089	1089-1107	CGAGGGCAAAUACAGCUU UTT	926	AAAGCUGUAUUUGCCCUC GTT	927
1096	1096-1114	AAAUACAGCUUUGGUGCC ATT	928	UGGCACCAAAGCUGUAUU UTT	929
1097	1097-1115	AAUACAGCUUUGGUGCCA CTT	930	GUGGCACCAAAGCUGUAU UTT	931
1098	1098-1116	AUACAGCUUUGGUGCCAC CTT	932	GGUGGCACCAAAGCUGUA UTT	933
1104	1104-1122	CUUUGGUGCCACCUGCGU GTT	934	CACGCAGGUGGCACCAAA GTT	935
1106	1106-1124	UUGGUGCCACCUGCGUGA ATT	936	UUCACGCAGGUGGCACCA ATT	937
1112	1112-1130	CCACCUGCGUGAAGAAGU GTT	938	CACUUCUUCACGCAGGUG GTT	939
1116	1116-1134	CUGCGUGAAGAAGUGUCC CTT	940	GGGACACUUCUUCACGCA GTT	941
1117	1117-1135	UGCGUGAAGAAGUGUCCC CTT	942	GGGGACACUUCUUCACGC ATT	943
1118	1118-1136	GCGUGAAGAAGUGUCCCC GTT	944	CGGGGACACUUCUUCACG CTT	945
1119	1119-1137	CGUGAAGAAGUGUCCCCG UTT	946	ACGGGGACACUUCUUCAC GTT	947
1120	1120-1138	GUGAAGAAGUGUCCCCGU ATT	948	UACGGGGACACUUCUUCA CTT	949
1121	1121-1139	UGAAGAAGUGUCCCCGUA ATT	950	UUACGGGGACACUUCUUC ATT	951
1122	1122-1140	GAAGAAGUGUCCCCGUAA UTT	952	AUUACGGGGACACUUCUU CTT	953
1123	1123-1141	AAGAAGUGUCCCCGUAAU UTT	954	AAUUACGGGGACACUUCU UTT	955
1124	1124-1142	AGAAGUGUCCCCGUAAUU ATT	956	UAAUUACGGGGACACUUC UTT	957
1125	1125-1143	GAAGUGUCCCCGUAAUUA UTT	958	AUAAUUACGGGGACACUU CTT	959
1126	1126-1144	AAGUGUCCCCGUAAUUAU GTT	960	CAUAAUUACGGGGACACU UTT	961
1127	1127-1145	AGUGUCCCCGUAAUUAUG UTT	962	ACAUAAUUACGGGGACAC UTT	963
1128	1128-1146	GUGUCCCCGUAAUUAUGU GTT	964	CACAUAAUUACGGGGACA CTT	965
1129	1129-1147	UGUCCCCGUAAUUAUGUG GTT	966	CCACAUAAUUACGGGGAC ATT	967
1130	1130-1148	GUCCCCGUAAUUAUGUGG UTT	968	ACCACAUAAUUACGGGGA CTT	969
1132	1132-1150	CCCCGUAAUUAUGUGGUG ATT	970	UCACCACAUAAUUACGGG GTT	971
1134	1134-1152	CCGUAAUUAUGUGGUGAC ATT	972	UGUCACCACAUAAUUACG GTT	973
1136	1136-1154	GUAAUUAUGUGGUGACAG ATT	974	UCUGUCACCACAUAAUUA CTT	975
1137	1137-1155	UAAUUAUGUGGUGACAGA UTT	976	AUCUGUCACCACAUAAUU ATT	977

1138	1138-1156	AAUUAUGUGGUGACAGAUCTT	978	GAUCUGUCACCACAUAAUUTT	979
1139	1139-1157	AUUAUGUGGUGACAGAUCATT	980	UGAUCUGUCACCACAUAAUTT	981
1140	1140-1158	UUAUGUGGUGACAGAUCACTT	982	GUGAUCUGUCACCACAUAATT	983
1142	1142-1160	AUGUGGUGACAGAUCACGGTT	984	CCGUGAUCUGUCACCACAUTT	985
1145	1145-1163	UGGUGACAGAUCACGGCUCTT	986	GAGCCGUGAUCUGUCACCATT	987
1147	1147-1165	GUGACAGAUCACGGCUCGUTT	988	ACGAGCCGUGAUCUGUCACTT	989
1148	1148-1166	UGACAGAUCACGGCUCGUGTT	990	CACGAGCCGUGAUCUGUCATT	991
1149	1149-1167	GACAGAUCACGGCUCGUGCTT	992	GCACGAGCCGUGAUCUGUCTT	993
1150	1150-1168	ACAGAUCACGGCUCGUGCGTT	994	CGCACGAGCCGUGAUCUGUTT	995
1151	1151-1169	CAGAUCACGGCUCGUGCGUTT	996	ACGCACGAGCCGUGAUCUGTT	997
1152	1152-1170	AGAUCACGGCUCGUGCGUCTT	998	GACGCACGAGCCGUGAUCUTT	999
1153	1153-1171	GAUCACGGCUCGUGCGUCCTT	1000	GGACGCACGAGCCGUGAUCTT	1001
1154	1154-1172	AUCACGGCUCGUGCGUCCGTT	1002	CGGACGCACGAGCCGUGAUTT	1003
1155	1155-1173	UCACGGCUCGUGCGUCCGATT	1004	UCGGACGCACGAGCCGUGATT	1005
1156	1156-1174	CACGGCUCGUGCGUCCGAGTT	1006	CUCGGACGCACGAGCCGUGTT	1007
1157	1157-1175	ACGGCUCGUGCGUCCGAGCTT	1008	GCUCGGACGCACGAGCCGUTT	1009
1160	1160-1178	GCUCGUGCGUCCGAGCCUGTT	1010	CAGGCUCGGACGCACGAGCTT	1011
1200	1200-1218	GGAGGAAGACGGCGUCCGCTT	1012	GCGGACGCCGUCUUCCUCCTT	1013
1201	1201-1219	GAGGAAGACGGCGUCCGCATT	1014	UGCGGACGCCGUCUUCCUCTT	1015
1203	1203-1221	GGAAGACGGCGUCCGCAAGTT	1016	CUUGCGGACGCCGUCUUCCTT	1017
1204	1204-1222	GAAGACGGCGUCCGCAAGUTT	1018	ACUUGCGGACGCCGUCUUCTT	1019
1205	1205-1223	AAGACGGCGUCCGCAAGUGTT	1020	CACUUGCGGACGCCGUCUUTT	1021
1207	1207-1225	GACGGCGUCCGCAAGUGUATT	1022	UACACUUGCGGACGCCGUCTT	1023
1208	1208-1226	ACGGCGUCCGCAAGUGUAATT	1024	UUACACUUGCGGACGCCGUTT	1025
1211	1211-1229	GCGUCCGCAAGUGUAAGAATT	1026	UUCUUACACUUGCGGACGCTT	1027
1212	1212-1230	CGUCCGCAAGUGUAAGAAGTT	1028	CUUCUUACACUUGCGGACGTT	1029
1213	1213-1231	GUCCGCAAGUGUAAGAAGUTT	1030	ACUUCUUACACUUGCGGACTT	1031
1214	1214-1232	UCCGCAAGUGUAAGAAGU	103	CACUUCUUACACUUGCGG	103

		GTT	2	ATT	3
1215	1215-1233	CCGCAAGUGUAAGAAGUGCTT	1034	GCACUUCUUACACUUGCGGTT	1035
1216	1216-1234	CGCAAGUGUAAGAAGUGCGTT	1036	CGCACUUCUUACACUUGCGTT	1037
1217	1217-1235	GCAAGUGUAAGAAGUGCGATT	1038	UCGCACUUCUUACACUUGCTT	1039
1219	1219-1237	AAGUGUAAGAAGUGCGAAGTT	1040	CUUCGCACUUCUUACACUUTT	1041
1220	1220-1238	AGUGUAAGAAGUGCGAAGGTT	1042	CCUUCGCACUUCUUACACUTT	1043
1221	1221-1239	GUGUAAGAAGUGCGAAGGGTT	1044	CCCUUCGCACUUCUUACACTT	1045
1222	1222-1240	UGUAAGAAGUGCGAAGGGCTT	1046	GCCCUUCGCACUUCUUACATT	1047
1223	1223-1241	GUAAGAAGUGCGAAGGGCCTT	1048	GGCCCUUCGCACUUCUUACTT	1049
1224	1224-1242	UAAGAAGUGCGAAGGGCCUTT	1050	AGGCCCUUCGCACUUCUUATT	1051
1225	1225-1243	AAGAAGUGCGAAGGGCCUUTT	1052	AAGGCCCUUCGCACUUCUUTT	1053
1226	1226-1244	AGAAGUGCGAAGGGCCUUGTT	1054	CAAGGCCCUUCGCACUUCUTT	1055
1229	1229-1247	AGUGCGAAGGGCCUUGCCGTT	1056	CGGCAAGGCCCUUCGCACUTT	1057
1230	1230-1248	GUGCGAAGGGCCUUGCCGCTT	1058	GCGGCAAGGCCCUUCGCACTT	1059
1231	1231-1249	UGCGAAGGGCCUUGCCGCATT	1060	UGCGGCAAGGCCCUUCGCATT	1061
1232	1232-1250	GCGAAGGGCCUUGCCGCAATT	1062	UUGCGGCAAGGCCCUUCGCTT	1063
1233	1233-1251	CGAAGGGCCUUGCCGCAAATT	1064	UUUGCGGCAAGGCCCUUCGTT	1065
1235	1235-1253	AAGGGCCUUGCCGCAAAGUTT	1066	ACUUUGCGGCAAGGCCCUUTT	1067
1236	1236-1254	AGGGCCUUGCCGCAAAGUGTT	1068	CACUUUGCGGCAAGGCCCUTT	1069
1237	1237-1255	GGGCCUUGCCGCAAAGUGUTT	1070	ACACUUUGCGGCAAGGCCCTT	1071
1238	1238-1256	GGCCUUGCCGCAAAGUGUGTT	1072	CACACUUUGCGGCAAGGCCTT	1073
1239	1239-1257	GCCUUGCCGCAAAGUGUGUTT	1074	ACACACUUUGCGGCAAGGCTT	1075
1241	1241-1259	CUUGCCGCAAAGUGUGUAATT	1076	UUACACACUUUGCGGCAAGTT	1077
1261	1261-1279	GGAAUAGGUAUUGGUGAAUTT	1078	AUUCACCAAUACCUAUUCCTT	1079
1262	1262-1280	GAAUAGGUAUUGGUGAAUUTT	1080	AAUUCACCAAUACCUAUUCTT	1081
1263	1263-1281	AAUAGGUAUUGGUGAAUUUTT	1082	AAAUUCACCAAUACCUAUUTT	1083
1264	1264-1282	AUAGGUAUUGGUGAAUUUATT	1084	UAAAUUCACCAAUACCUAUTT	1085
1266	1266-1284	AGGUAUUGGUGAAUUUAAATT	1086	UUUAAAUUCACCAAUACCUTT	1087

1267	1267-1285	GGUAUUGGUGAAUUUAAAGTT	1088	CUUUAAAUUCACCAAUACCTT	1089
1289	1289-1307	CACUCUCCAUAAAUGCUACTT	1090	GUAGCAUUUAUGGAGAGUGTT	1091
1313	1313-1331	UUAAACACUUCAAAAACUGTT	1092	CAGUUUUUGAAGUGUUUAATT	1093
1320	1320-1338	CUUCAAAAACUGCACCUCCTT	1094	GGAGGUGCAGUUUUUGAAGTT	1095
1321	1321-1339	UUCAAAAACUGCACCUCCATT	1096	UGGAGGUGCAGUUUUUGAATT	1097
1322	1322-1340	UCAAAAACUGCACCUCCAUTT	1098	AUGGAGGUGCAGUUUUUGATT	1099
1323	1323-1341	CAAAAACUGCACCUCCAUCTT	1100	GAUGGAGGUGCAGUUUUUGTT	1101
1324	1324-1342	AAAAACUGCACCUCCAUCATT	1102	UGAUGGAGGUGCAGUUUUUTT	1103
1328	1328-1346	ACUGCACCUCCAUCAGUGGTT	1104	CCACUGAUGGAGGUGCAGUTT	1105
1332	1332-1350	CACCUCCAUCAGUGGCGAUTT	1106	AUCGCCACUGAUGGAGGUGTT	1107
1333	1333-1351	ACCUCCAUCAGUGGCGAUCTT	1108	GAUCGCCACUGAUGGAGGUTT	1109
1335	1335-1353	CUCCAUCAGUGGCGAUCUCTT	1110	GAGAUCGCCACUGAUGGAGTT	1111
1338	1338-1356	CAUCAGUGGCGAUCUCCACTT	1112	GUGGAGAUCGCCACUGAUGTT	1113
1344	1344-1362	UGGCGAUCUCCACAUCCUGTT	1114	CAGGAUGUGGAGAUCGCCATT	1115
1345	1345-1363	GGCGAUCUCCACAUCCUGCTT	1116	GCAGGAUGUGGAGAUCGCCTT	1117
1346	1346-1364	GCGAUCUCCACAUCCUGCCTT	1118	GGCAGGAUGUGGAGAUCGCTT	1119
1347	1347-1365	CGAUCUCCACAUCCUGCCGTT	1120	CGGCAGGAUGUGGAGAUCGTT	1121
1348	1348-1366	GAUCUCCACAUCCUGCCGGTT	1122	CCGGCAGGAUGUGGAGAUCTT	1123
1353	1353-1371	CCACAUCCUGCCGGUGGCATT	1124	UGCCACCGGCAGGAUGUGGTT	1125
1354	1354-1372	CACAUCCUGCCGGUGGCAUTT	1126	AUGCCACCGGCAGGAUGUGTT	1127
1355	1355-1373	ACAUCCUGCCGGUGGCAUUTT	1128	AAUGCCACCGGCAGGAUGUTT	1129
1357	1357-1375	AUCCUGCCGGUGGCAUUUATT	1130	UAAAUGCCACCGGCAGGAUTT	1131
1360	1360-1378	CUGCCGGUGGCAUUUAGGGTT	1132	CCCUAAAUGCCACCGGCAGTT	1133
1361	1361-1379	UGCCGGUGGCAUUUAGGGGTT	1134	CCCCUAAAUGCCACCGGCATT	1135
1362	1362-1380	GCCGGUGGCAUUUAGGGGUTT	1136	ACCCCUAAAUGCCACCGGCTT	1137
1363	1363-1381	CCGGUGGCAUUUAGGGGUGTT	1138	CACCCCUAAAUGCCACCGGTT	1139
1366	1366-1384	GUGGCAUUUAGGGGUGACUTT	1140	AGUCACCCCUAAAUGCCACTT	1141
1369	1369-1387	GCAUUUAGGGGUGACUCC	114	AGGAGUCACCCCUAAAUG	114

		UTT	2	CTT	3
1370	1370-1388	CAUUUAGGGGUGACUCCUUTT	1144	AAGGAGUCACCCCUAAAUGTT	1145
1371	1371-1389	AUUUAGGGGUGACUCCUUCTT	1146	GAAGGAGUCACCCCUAAAUTT	1147
1372	1372-1390	UUUAGGGGUGACUCCUUCATT	1148	UGAAGGAGUCACCCCUAAATT	1149
1373	1373-1391	UUAGGGGUGACUCCUUCACTT	1150	GUGAAGGAGUCACCCCUAATT	1151
1374	1374-1392	UAGGGGUGACUCCUUCACATT	1152	UGUGAAGGAGUCACCCCUATT	1153
1404	1404-1422	UCUGGAUCCACAGGAACUGTT	1154	CAGUUCCUGUGGAUCCAGATT	1155
1408	1408-1426	GAUCCACAGGAACUGGAUATT	1156	UAUCCAGUUCCUGUGGAUCTT	1157
1409	1409-1427	AUCCACAGGAACUGGAUAUTT	1158	AUAUCCAGUUCCUGUGGAUTT	1159
1411	1411-1429	CCACAGGAACUGGAUAUUCTT	1160	GAAUAUCCAGUUCCUGUGGTT	1161
1412	1412-1430	CACAGGAACUGGAUAUUCUTT	1162	AGAAUAUCCAGUUCCUGUGTT	1163
1419	1419-1437	ACUGGAUAUUCUGAAAACCTT	1164	GGUUUUCAGAAUAUCCAGUTT	1165
1426	1426-1444	AUUCUGAAAACCGUAAAGGTT	1166	CCUUUACGGUUUUCAGAAUTT	1167
1427	1427-1445	UUCUGAAAACCGUAAAGGATT	1168	UCCUUUACGGUUUUCAGAATT	1169
1430	1430-1448	UGAAAACCGUAAAGGAAAUTT	1170	AUUUCCUUUACGGUUUUCATT	1171
1431	1431-1449	GAAAACCGUAAAGGAAAUCTT	1172	GAUUUCCUUUACGGUUUUCTT	1173

Table 5. AR Target Sequences

ID	Code	Target Sequence	SEQ ID NO:	NM_000044.3	Exon	Species
XD-01817K1	**17**	**CAAAGGUUCUCUGCUAGACGACA**	**1174**	**1987-2005**	**1**	**h**
XD-01827K1	**27**	**UCUGGGUGUCACUAUGGAGCUCU**	**1175**	**2819-2837**	**2**	**h**
XD-01828K1	**28**	**CUGGGUGUCACUAUGGAGCUCUC**	**1176**	**2820-2838**	**2**	**h**
XD-01829K1	**29**	**GGGUGUCACUAUGGAGCUCUCAC**	**1177**	**2822-2840**	**2**	**h**
XD-01821K1	21	UACUACAACUUUCCACUGGCUCU	1178	2207-2225	1	h
XD-01825K1	25	AAGCUUCUGGGUGUCACUAUGGA	1179	2814-2832	2	h,m
XD-01826K1	26	CUUCUGGGUGUCACUAUGGAGCU	1180	2817-2835	2	h

Table 6. β-catenin Target Sequences

R #	Generic	Gene	Target sequences

	name					
R-1146	1797mfm	CTNNB1	CUGUUGGAUUGAUUCGAAAUU	SEQ ID NO: 1181	UUUCGAAUCAAUCCAACAGUU	SEQ ID NO: 1182
R-1147	1870mfm	CTNNB1	ACGACUAGUUCAGUUGCUUUU	SEQ ID NO: 1183	AAGCAACUGAACUAGUCGUUU	SEQ ID NO: 1184

Table 7. PIK3CA* and PIK3CB* Target Sequences

Gene Symbol	Gene ID	Name	Target Sequences (97-mer)	SEQ ID NO:
PIK3CA	5290	PIK3CA_1746	TGCTGTTGACAGTGAGCGCCAGCTCAAAGCAATTTCTACATAGTGAAGCCACAGATGTATGTAGAAATTGCTTTGAGCTGTTGCCTACTGCCTCGGA	1185
PIK3CA	5290	PIK3CA_2328	TGCTGTTGACAGTGAGCGAAAGGATGAAACACAAAAGGTATAGTGAAGCCACAGATGTATACCTTTTGTGTTTCATCCTTCTGCCTACTGCCTCGGA	1186
PIK3CA	5290	PIK3CA_2522	TGCTGTTGACAGTGAGCGCCATGTCAGAGTTACTGTTTCATAGTGAAGCCACAGATGTATGAAACAGTAACTCTGACATGATGCCTACTGCCTCGGA	1187
PIK3CA	5290	PIK3CA_3555	TGCTGTTGACAGTGAGCGCAACTAGTTCATTTCAAAATTATAGTGAAGCCACAGATGTATAATTTTGAAATGAACTAGTTTTGCCTACTGCCTCGGA	1188
PIK3CA	5290	PIK3CA_3484	TGCTGTTGACAGTGAGCGCACAGCAAGAACAGAAATAAAATAGTGAAGCCACAGATGTATTTTATTTCTGTTCTTGCTGTATGCCTACTGCCTCGGA	1189
PIK3CB	5291	PIK3CB_862	TGCTGTTGACAGTGAGCGACAAGATCAAGAAAATGTATGATAGTGAAGCCACAGATGTATCATACATTTTCTTGATCTTGCTGCCTACTGCCTCGGA	1190
PIK3CB	5291	PIK3CB_183	TGCTGTTGACAGTGAGCGCAGCAAGTTCACAATTACCCAATAGTGAAGCCACAGATGTATTGGGTAATTGTGAACTTGCTTTGCCTACTGCCTCGGA	1191
PIK3CB	5291	PIK3CB_1520	TGCTGTTGACAGTGAGCGCCCCTTCGATAAGATTATTGAATAGTGAAGCCACAGATGTATTCAATAATCTTATCGAAGGGATGCCTACTGCCTCGGA	1192
PIK3CB	5291	PIK3CB_272	TGCTGTTGACAGTGAGCGAGAGCTTGAAGATGAAACACGATAGTGAAGCCACAGATGTATCGTGTTTCATCTTCAAGCTCCTGCCTACTGCCTCGGA	1193
PIK3CB	5291	PIK3CB_948	TGCTGTTGACAGTGAGCGACACCAAAGAAAACACGAATTATAGTGAAGCCACAGATGTATAATTCGTGTTTTCTTTGGTGGTGCCTACTGCCTCGGA	1194

*Species is Homo sapiens.

Table 8. PIK3CA and PIK3CB siRNA Sequences

Gene Symbol	Gene ID	Name	siRNA Guide	SEQ ID NO:	siRNA passenger	SEQ ID NO:
PIK3CA	5290	PIK3CA_1746	UGUAGAAAUUGCUUUGAGCUGU	1195	AGCUCAAAGCAAUUUCUACAUA	1196
PIK3CA	5290	PIK3CA_2328	UACCUUUUGUGUUUCAUCCUUC	1197	AGGAUGAAACACAAAAGGUAUA	1198
PIK3CA	5290	PIK3CA_2522	UGAAACAGUAACUCUGACAUGA	1199	AUGUCAGAGUUACUGUUUCAUA	1200
PIK3CA	5290	PIK3CA_3555	UAAUUUUGAAAUGAACUAGUUU	1201	ACUAGUUCAUUUCAAAAUUAUA	1202

PIK3CA	5290	PIK3CA_3484	UUUUAUUUCUGUUCUUGCUGUA	1203	CAGCAAGAACAGAAAUAAAAUA	1204
PIK3CB	5291	PIK3CB_862	UCAUACAUUUUCUUGAUCUUGC	1205	AAGAUCAAGAAAAUGUAUGAUA	1206
PIK3CB	5291	PIK3CB_183	UUGGGUAAUUGUGAACUUGCUU	1207	GCAAGUUCACAAUUACCCAAUA	1208
PIK3CB	5291	PIK3CB_1520	UUCAAUAAUCUUAUCGAAGGGA	1209	CCUUCGAUAAGAUUAUUGAAUA	1210
PIK3CB	5291	PIK3CB_272	UCGUGUUUCAUCUUCAAGCUCC	1211	AGCUUGAAGAUGAAACACGAUA	1212
PIK3CB	5291	PIK3CB_948	UAAUUCGUGUUUUCUUUGGUGG	1213	ACCAAAGAAAACACGAAUUAUA	1214

Table 9. Additional polynucleic acid molecule sequences

	Base start position	**Guide strand**	**SEQ ID NO:**	**Passenger strand**	**SEQ ID NO:**
EGFR R1246	333	ACUCGUGCCUUGGCAAACUUU	1215	AGUUUGCCAAGGCACGAGUUU	1216
EGFR R1195	333	ACUCGUGCCUUGGCAAACUUU	1217	AGUUUGCCAAGGCACGAGUUU	1218
EGFR R1449	333	ACUCGUGCCUUGGCAAACUUU	1219	AGUUUGCCAAGGCACGAGUUU	1220
KRAS R1450	237	UGAAUUAGCUGUAUCGUCAUU	1221	TGACGAUACAGCUAAUUCAUU	1222
KRAS R1443	237	UGAAUUAGCUGUAUCGUCAUU	1223	UGACGAUACAGCUAAUUCAUU	1224
KRAS R1194	237	UGAAUUAGCUGUAUCGUCAUU	1225	UGACGAUACAGCUAAUUCAUU	1226
CTNNB1 R1442	1248	UAAGUAUAGGUCCUCAUUAUU	1227	UAAUGAGGACCUAUACUUAUU	1228
CTNNB1 R1404	1797	TUUCGAAUCAAUCCAACAGUU	1229	CUGUUGGAUUGAUUCGAAAUU	1230
CTNNB1 R1441	1797	UUUCGAAUCAAUCCAACAGUU	1231	CUGUUGGAUUGAUUCGAAAUU	1232
CTNNB1 R1523	1797	UUUCGAAUCAAUCCAACAGUU	1233	CUGUUGGAUUGAUUCGAAAUU	1234
HPRT R1492	425	AUAAAAUCUACAGUCAUAGUU	1235	CUAUGACUGUAGAUUUUAUUU	1236
HPRT R1526	425	UUAAAAUCUACAGUCAUAGUU	1237	CUAUGACUGUAGAUUUUAAUU	1238
HPRT R1527	425	UUAAAAUCUACAGUCAUAGUU	1239	CUAUGACUGUAGAUUUUAAUU	1240
AR R1245	2822	GAGAGCUCCAUAGUGACACUU	1241	GUGUCACUAUGGAGCUCUCUU	1242

Example 2. General Experimental Protocol

[0473] Stem-loop qPCR assay for quantification of siRNA

[0474] Plasma samples are directly diluted in TE buffer. 50 mg tissue pieces are homogenized in 1 mL of Trizol using a FastPrep-24 tissue homogenizer (MP Biomedicals) and then diluted in TE buffer. Standard curves are generated by spiking siRNA into plasma or homogenized tissue from untreated

animals and then serially diluting with TE buffer. The antisense strand of the siRNA is reverse transcribed using a TaqMan MicroRNA reverse transcription kit (Applied Biosystems) with 25 nM of a sequence-specific stem-loop RT primer. The cDNA from the RT step is utilized for real-time PCR using TaqMan Fast Advanced Master Mix (Applied Biosystems) with 1.5 μM of forward primer, 0.75 μM of reverse primer, and 0.2 μM of probe. Quantitative PCR reactions are performed using standard cycling conditions in a ViiA 7 Real-Time PCR System (Life Technologies). The Ct values are transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

[0475] Comparative qPCR assay for determination of mRNA knockdown

[0476] Tissue samples are homogenized in Trizol as described above. Total RNA is isolated using RNeasy RNA isolation 96-well plates (Qiagen), then 500 ng RNA is reverse transcribed with a High Capacity RNA to cDNA kit (ThermoFisher). KRAS, EGFR, CTNNB1, and PPIB mRNAs are quantified by TaqMan qPCR analysis performed with a ViiA 7 Real-Time PCR System. The TaqMan primers and probes for EGFR and CTNNB1 are purchased from Applied Biosystems as pre-validated gene expression assays. PPIB (housekeeping gene) is used as an internal RNA loading control, with all TaqMan primers and probes for PPIB purchased from Applied Biosystems as pre-validated gene expression assays. Results are calculated by the comparative Ct method, where the difference between the target gene (KRAS, CTNNB1, or EGFR) Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt).

[0477] Animals

[0478] All animal studies are conducted following protocols in accordance with the Institutional Animal Care and Use Committee (IACUC) at Explora BioLabs, which adhere to the regulations outlined in the USDA Animal Welfare Act as well as the "Guide for the Care and Use of Laboratory Animals" (National Research Council publication, 8th Ed., revised in 2011). All mice are obtained from either Charles River Laboratories or Harlan Laboratories.

[0479] H358, HCC827, and Hep-3B2 1-7 subcutaneous flank tumor model

[0480] For the H358 subcutaneous flank tumor model, tumor cells are inoculated and tumors are established according to the following methods. Female NCr nu/nu mice are identified by ear-tag the day before cell injection. Mice are weighed prior to inoculation. H358 cells are cultured with 10% FBS/RPMI medium and harvested with 0.05% Trypsin and Cell Stripper (MediaTech). 5 million H358 cells in 0.05 ml Hank's Balanced Salt Solution (HBSS) with Matrigel (1:1) are injected subcutaneously (SC) into the upper right flank of each mouse. Tumor growth is monitored by tumor volume measurement using a digital caliper starting on day 7 after inoculation, and followed 2 times per week until average tumor volume reaches >100 & ≤ 300 mm^3. Once tumors are to the desired volume (average from 100 to 300 mm^3), animals are randomized and mice with very large or small tumors are culled. Mice are divided into the required groups and randomized by tumor volume. Mice are then treated as described in the individual experiments.

[0481] For the Hep3B orthotropic liver tumor model, tumor cells are inoculated and tumors are established according to the following methods. Female NCr nu/nu mice are identified by ear-tag the day

before, mice will be anesthetized with isoflurane. The mice are then placed in a supine position on a water circulating heating pad to maintain body temperature. A small transverse incision below the sternum will be made to expose the liver. Cancer cells are slowly injected into the upper left lobe of the liver using a 28-gauge needle. The cells are injected at a 30-degree angle into the liver, so that a transparent bleb of cells can be seen through the liver capsule. Hep 3B2.1 7 cells are prepared by suspending in cold PBS ($0.1\text{-}5x10^6$ cells) and mixing with diluted matrigel (30x in PBS). 30-50 ul of the cell/matrigel is inoculated. After injection, a small piece of sterile gauze is placed on the injection site, and light pressure was applied for 1 min to prevent bleeding. The abdomen is then closed with a 6-0 silk suture. After tumor cell implantation, animals are kept in a warm cage, observed for 1–2 h, and subsequently returned to the animal room after full recovery from the anesthesia. 7-10 days after tumor implantation animals are randomized, divided into the required groups and then treated as described in the individual experiments.

[0482] LNCap subcutaneous flank tumor model

[0483] LNCaP cells (ATCC® CRL-1740™) are grown in RPMI + 10% FBS supplemented with non-essential amino acids and sodium pyruvate to a confluency of about 80%. Cells are mixed 1:1 with matrigel and 5-7*106 cells injected subcutaneously into male SCID mice (6-8 weeks). Tumors are usually developed within 3-5 weeks to a size of 100-350 mm^3. Animals bearing tumors within this range are randomized and treated with ASCs by injections into the tail vein. For PD studies animals are sacrificed 96 hours after injection and organ fragments harvested, weighted, and frozen in liquid nitrogen. For RNA isolation, organ samples are homogenized in Trizol and RNA prepared using a Qiagen RNeasy 96 Plus kit following the instructions by the manufacturer. RNA concentrations are determined spectroscopically. RNAs are converted into cDNAs by reverse transcription and expression of specific targets quantified by qPCR using the ΔΔCT method and validated Taqman assays (Thermofisher). Samples are standardize to the expression levels of PPIB.

[0484] Peptide synthesis

[0485] Peptides are synthesized on solid phase using standard Fmoc chemistry. Both peptides have cysteine at the N-terminus and the cleaved peptides are purified by HPLC and confirmed by mass spectroscopy. INF7 peptide is as illustrated SEQ ID NO: 2055. Melittin peptide is as illustrated SEQ ID NO: 2060.

[0486] Anti-EGFR Antibody

[0487] Anti-EGFR antibody is a fully human IgG1κ monoclonal antibody directed against the human epidermal growth factor receptor (EGFR). It is produced in the Chinese Hamster Ovary cell line DJT33, which has been derived from the CHO cell line CHO-K1SV by transfection with a GS vector carrying the antibody genes derived from a human anti-EGFR antibody producing hybridoma cell line (2F8). Standard mammalian cell culture and purification technologies are employed in the manufacturing of anti-EGFR antibody.

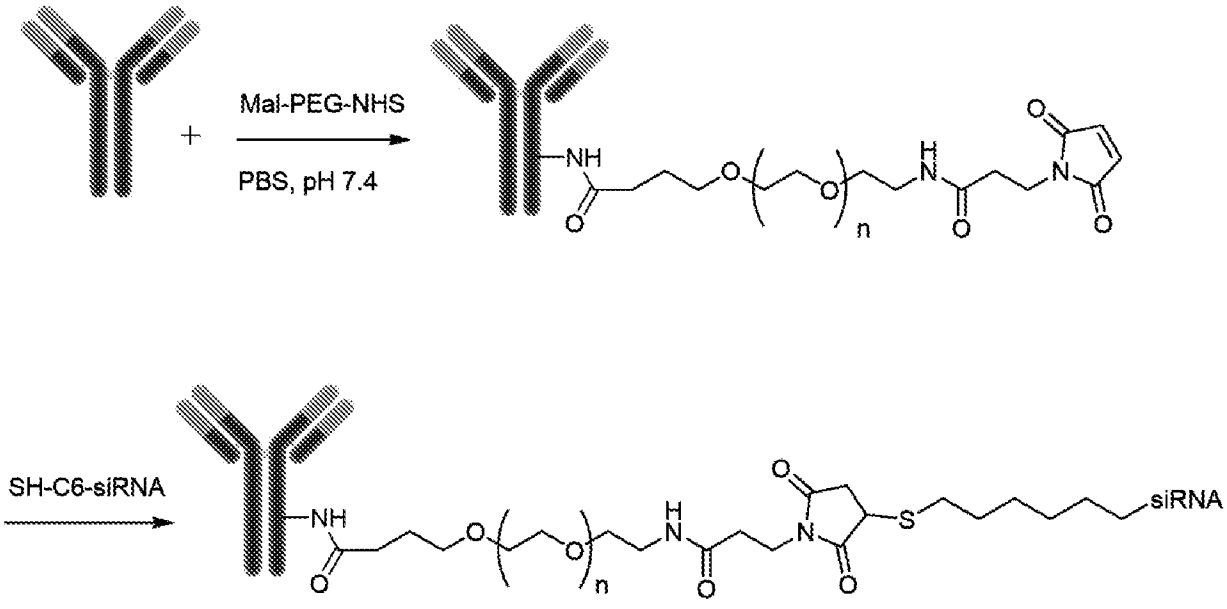
[0488] The theoretical molecular weight (MW) of anti-EGFR antibody without glycans is 146.6 kDa. The experimental MW of the major glycosylated isoform of the antibody is 149 kDa as determined by

mass spectrometry. Using SDS-PAGE under reducing conditions the MW of the light chain was found to be approximately 25 kDa and the MW of the heavy chain to be approximately 50 kDa. The heavy chains are connected to each other by two inter-chain disulfide bonds, and one light chain is attached to each heavy chain by a single inter-chain disulfide bond. The light chain has two intra-chain disulfide bonds and the heavy chain has four intra-chain disulfide bonds. The antibody is N-linked glycosylated at Asn305 of the heavy chain with glycans composed of N-acetyl-glucosamine, mannose, fucose and galactose. The predominant glycans present are fucosylated bi-antennary structures containing zero or one terminal galactose residue.

[0489] The charged isoform pattern of the IgG1κ antibody has been investigated using imaged capillary IEF, agarose IEF and analytical cation exchange HPLC. Multiple charged isoforms are found, with the main isoform having an isoelectric point of approximately 8.7.

[0490] The major mechanism of action of anti-EGFR antibody is a concentration dependent inhibition of EGF-induced EGFR phosphorylation in A431 cancer cells. Additionally, induction of antibody-dependent cell-mediated cytotoxicity (ADCC) at low antibody concentrations has been observed in pre-clinical cellular in vitro studies.

Example 3: Synthesis, purification and analysis of antibody-PEG-EGFR and antibody-EGFR conjugates



Conjugation scheme-1

[0491] <u>**Step 1: Antibody conjugation with maleimide-PEG-NHS followed by SH-EGFR**</u>

[0492] Anti-EGFR antibody (EGFR-Ab) is exchanged with 1X Phosphate buffer (pH 7.4) and made up to 5mg/ml concentration. To this solution, 2 equivalents of SMCC linker or maleimide-PEGxkDa-NHS (x = 1, 5, 10, 20) is added and rotated for 4 hours at room temperature. Unreacted maleimide-PEG is removed by spin filtration using 50 kDa MWCO Amicon spin filters and PBS pH 7.4. The antibody-PEG-Mal conjugate is collected and transferred into a reaction vessel. SH-C6-EGFR (2 equivalents) is

added at RT to the antibody-PEG-maleimide in PBS and rotated overnight. The reaction mixture is analyzed by analytical SAX column chromatography and conjugate along with unreacted antibody and siRNA is seen.

[0493] **Step 2: Purification**

[0494] The crude reaction mixture is purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing the antibody-PEG-EGFR conjugate ae pooled, concentrated and buffer exchanged with PBS, pH 7.4. Antibody siRNA conjugates with SMCC linker, PEG1kDa, PEG5kDa and PEG10kDa are separated based on the siRNA loading.

[0495] **Step-3: Analysis of the purified conjugate**

The isolated conjugate ias characterized by either mass spec or SDS-PAGE. The purity of the conjugate is assessed by analytical HPLC using either anion exchange chromatography method-2 or anion exchange chromatography method-3.

[0496] **Anion exchange chromatography method-1**

1. Column: Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 um
2. Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0; Flow Rate: 6.0 ml/min
3. Gradient:

	%A	%B	Column Volume
a.			
b.	100	0	1.00
c.	60	40	18.00
d.	40	60	2.00
e.	40	60	5.00
f.	0	100	2.00
g.	100	0	2.00

[0497] **Anion exchange chromatography method-2**

1. Column: Thermo Scientific, ProPac™ SAX-10, Bio LC™, 4 X 250 mm
2. Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl; Flow Rate: 1.0 ml/min
3. Gradient:

	Time	%A	%B
a.			
b.	0.0	90	10
c.	3.00	90	10
d.	11.00	40	60
e.	13.00	40	60
f.	15.00	90	10
g.	20.00	90	10

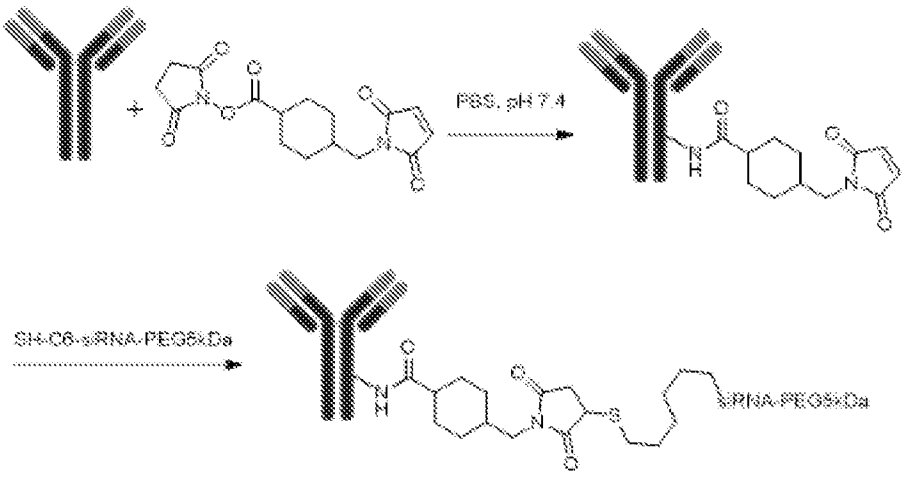
[0498] **Anion exchange chromatography method-3**

1. Column: Thermo Scientific, ProPacTM SAX-10, Bio LCTM, 4 X 250 mm
2. Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl
3. Flow Rate: 0.75 ml/min
4. Gradient:

	Time	%A	%B
a.			
b.	0.0	90	10
c.	3.00	90	10
d.	11.00	40	60
e.	23.00	40	60

f.	25.00	90	10
g.	30.00	90	10

Example 4: Synthesis, purification and analysis of antibody-siRNA-PEG conjugates



Conjugation Scheme-2

[0499] **Step 1: Antibody conjugation with SMCC linker followed by SH-KRAS-PEG5kDa**

[0500] Anti-EGFR antibody is exchanged with 1X Phosphate buffer (pH 7.4) and made up to 5mg/ml concentration. To this solution, 2 equivalents of SMCC linker (succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) is added and rotated for 4 hours at room temperature. Unreacted SMCC linker is removed by spin filtration using 50 kDa MWCO Amicon spin filters and PBS buffer pH 7.4. The retentate is collected and 2 equivalents of SH-C6-KRAS-PEG5kDa is added at RT and rotated overnight. The reaction mixture is analyzed by analytical SAX column chromatography.

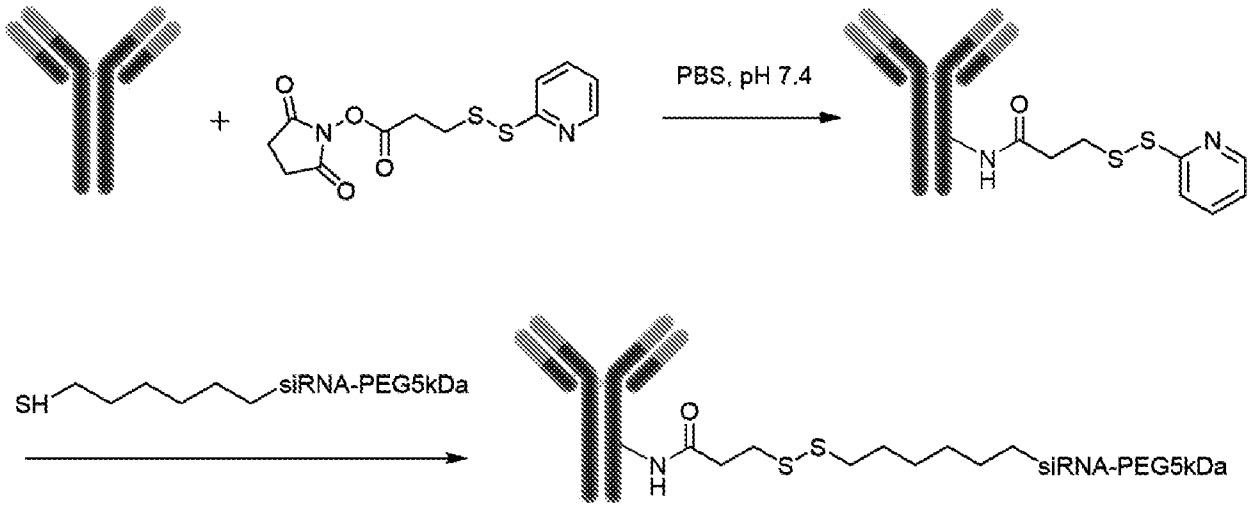
[0501] **Step 2: Purification**

[0502] The crude reaction mixture is purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing the antibody-KRAS-PEG conjugate are pooled, concentrated and buffer exchanged with PBS, pH 7.4.

[0503] **Step-3: Analysis of the purified conjugate**

The isolated conjugate is characterized by either mass spec or SDS-PAGE. The purity of the conjugate is assessed by analytical HPLC using anion exchange chromatography method-3 (described in example 1).

Example 5: Synthesis, purification and analysis of antibody-S-S-siRNA-PEG conjugates



Conjugation scheme-3

[0504] **Step 1: Antibody conjugation with SPDP linker followed by SH-siRNA-PEG5kDa**

[0505] Anti-EGFR antibody is exchanged with 1X Phosphate buffer (pH 7.4) and made up to 5mg/ml concentration. To this solution, 2 equivalents of SPDP linker (succinimidyl 3-(2-pyridyldithio)propionate) is added and rotated for 4 hours at room temperature. Unreacted SPDP linker is removed by spin filtration using 50 kDa MWCO Amicon spin filters and pH 7.4 PBS buffer. The retentate is collected and 2 equivalents of SH-C6-siRNA-PEG5kDa is added at room temperature and rotated overnight. The reaction mixture is analyzed by analytical SAX column chromatography and conjugate along with unreacted antibody determined.

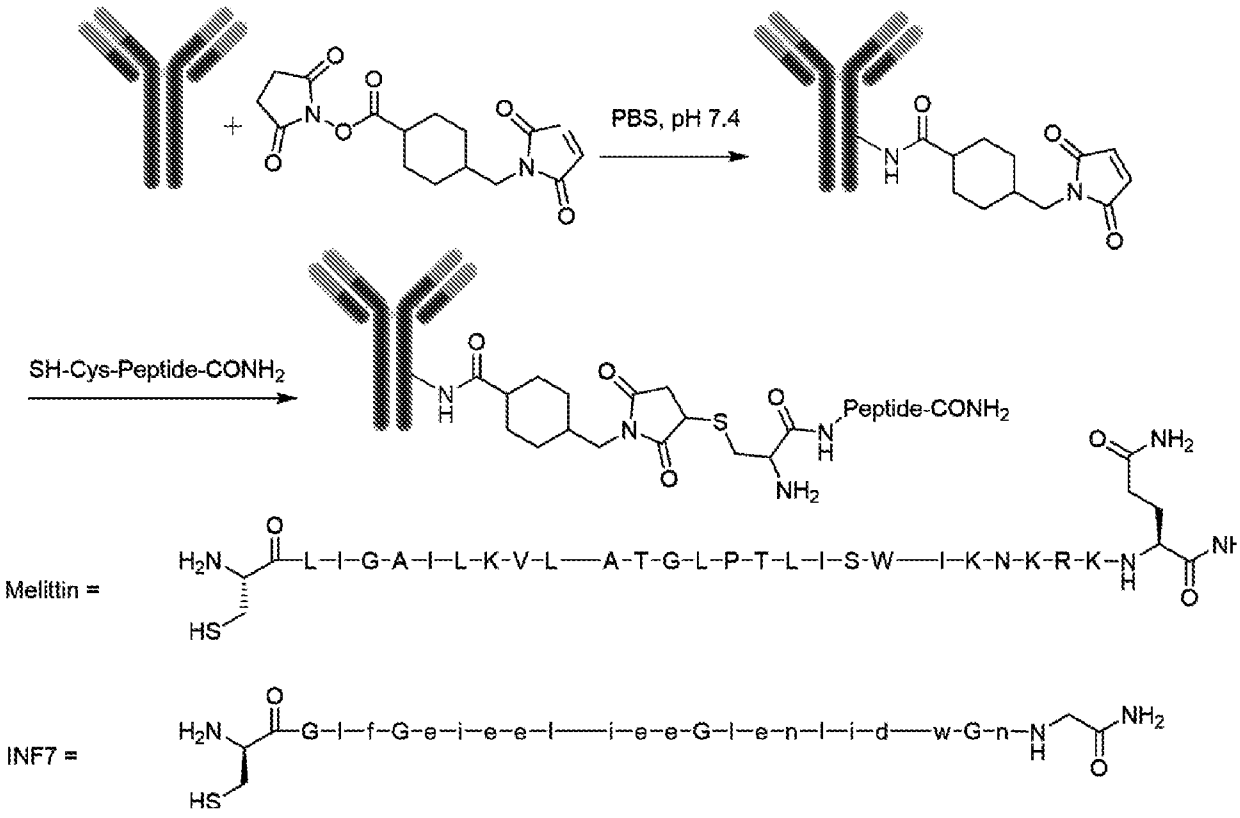
[0506] **Step 2: Purification**

[0507] The crude reaction mixture is purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing the antibody-PEG-siRNA conjugate are pooled, concentrated and buffer exchanged with PBS, pH 7.4.

[0508] **Step-3: Analysis of the purified conjugate**

The isolated conjugate is characterized by either mass spec or SDS-PAGE. The purity of the conjugate is assessed by analytical HPLC using anion exchange chromatography method-2.

Example 6: Synthesis, purification and analysis of antibody-SMCC-Endosomal escape peptide conjugates



Conjugation Scheme-4

[0509] **Step 1: Antibody conjugation with SMCC linker or maleimide-PEG-NHS followed by SH-Cys-Peptide-CONH$_2$**

[0510] Anti-EGFR antibody is exchanged with 1X Phosphate buffer (pH 7.4) and made up to 10mg/ml concentration. To this solution, 3 equivalents of SMCC linker (succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) or maleimide-PEG1kDa-NHS is added and rotated for 1.5 hours at room temperature. Unreacted SMCC linker or PEG linker is removed by spin filtration using 50 kDa MWCO Amicon spin filters and PBS buffer pH 7.4 (25mM MES pH=6.1 for Melittin conjugates). The retentate is collected and 3 equivalents of SH-Cys-Peptide-CONH$_2$ is added at RT and rotated overnight. The reaction mixture is then purified by either HIC chromatography or cation exchange chromatography to isolate the anti-EGFR antibody-Peptide or anti-EGFR antibody-PEG1k-Peptide.

[0511] **Step 2: Purification**

[0512] The crude reaction mixture is purified by AKTA explorer FPLC using either hydrophobic interaction chromatography (HIC) method-1 or cation exchange chromatography method-1. Fractions containing the antibody-peptide conjugates are pooled, concentrated and buffer exchanged with PBS, pH 7.4 (10 mM Acetate pH=6.0 for Melittin conjugates).

[0513] **Step-3: Analysis of the purified conjugate**

The isolated conjugate is characterized by either mass spec or SDS-PAGE. Purity and peptide loading is assessed by analytical HPLC using either HIC method-2 or cation exchange chromatography method-2.

[0514] **Cation exchange chromatography method-1**

1. Column: GE Healthcare HiPrep SP HP 16/10
2. Solvent A: 50 mM MES pH=6.0; Solvent B: 50 mM MES + 0.5M NaCl pH=6.0; Flow Rate: 2.0 ml/min
3. Gradient:

	%A	%B	Column Volume
b.	100	0	0.1
c.	100	0	Flush loop 12ml
d.	100	0	2.5
e.	0	100	15
f.	0	100	5
g.	100	0	0.5
h.	100	0	5

[0515] **Cation exchange chromatography method -2**

1. Column: Thermo Scientific, MAbPac™ SCX-10, Bio LC™, 4 X 250 mm (product # 074625)
2. Solvent A: 20 mM MES pH=5.5; Solvent B: 20 mM MES + 0.3 M NaCl pH=5.5; Flow Rate: 0.5 ml/min
3. Gradient:

	Time	%A	%B
b.	0.0	100	0
c.	5	100	0
d.	50	0	100
e.	80	0	100
f.	85	100	0
g.	90	100	0

[0516] **Hydrophobic interaction chromatography method-1 (HIC method-1)**

1. Column: GE Healthcare Butyl Sepharose High Performance (17-5432-02) 200ml
2. Solvent A: 50 mM Sodium Phosphate + 0.8M ammonium sulfate (pH=7.0); Solvent B: 80% 50 mM Sodium Phosphate (pH=7.0), 20% IPA; Flow Rate: 3.0 ml/min
3. Gradient:

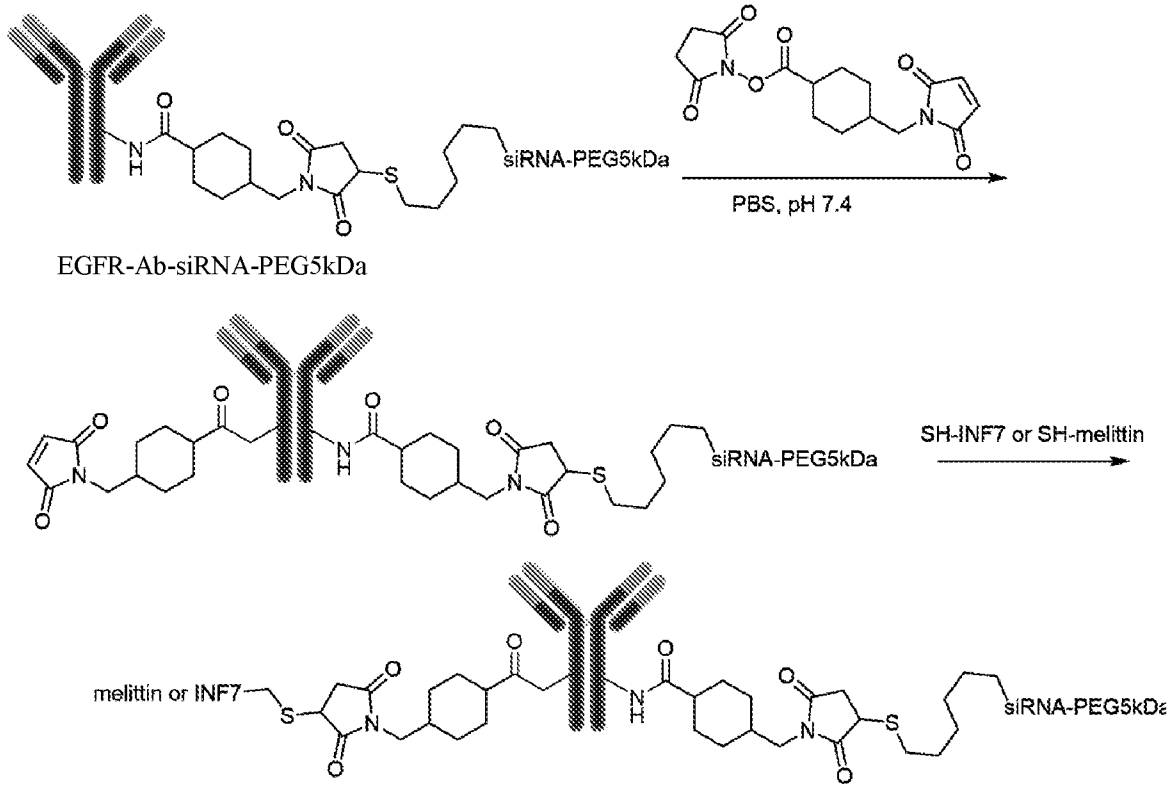
	%A	%B	Column Volume
b.	100	0	0.1
c.	0	100	3
d.	0	100	1.35
e.	100	0	0.1
f.	100	0	0.5

[0517] **Hydrophobic interaction chromatography method-2 (HIC method-2)**

1. Column: Tosoh Bioscience TSKgel Butyl-NPR 4.6mm ID x 10cm 2.5 µm
2. Solvent A: 100 mM Sodium phosphate + 1.8 M ammonium sulfate (pH=7.0); Solvent B: 80% 100 mM sodium phosphate (pH=7.0), 20% IPA; Flow Rate: 0.5 ml/min
3. Gradient:

	Time	%A	%B
b.	0	100	0
c.	3	50	50
d.	21	0	100
e.	23	0	100
f.	25	100	0

Example 7: Synthesis, purification and analysis of EEP-antibody-siRNA-PEG conjugates



Conjugation Scheme-5

[0518] **Step 1: Conjugation of PEG24 linker followed by SH-Cys-Peptide-$CONH_2$ to EGFR-Ab-siRNA-PEG**

[0519] EGFR-Ab-siRNA-PEG conjugate with a siRNA loading of 1 is conjugated with 4 equivalents of PEG1k linker (succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) in PBS, pH 7.4 buffer and rotated for 1.5 hours at room temperature. Unreacted PEG1k linker is removed by spin filtration using 50 kDa MWCO Amicon spin filters and PBS buffer pH 7.4. The retentate is collected and 4 equivalents of SH-Cys-Peptide-$CONH_2$ is added at RT and rotated overnight.

[0520] **Step 2: Purification**

[0521] The reaction mixture is then purified by repeated spin filtration using PBS buffer pH7.4 and 50 kDa Amicon spin filters until the unreacted peptide is removed as monitored by HPLC. The product contains a mixture of conjugates with 0, 1, 2, 3 or more peptides conjugated to the antibody backbone.

[0522] **Step-3: Analysis of the purified conjugate**

The isolated conjugate is characterized by either mass spec or SDS-PAGE. The purity and the peptide loading of the conjugate is assessed by analytical HPLC using either HIC method-2 or cation exchange chromatography method-2.

Example 8. Tumor PK/PD Study

[0523] Female NCr nu/nu mice bearing subcutaneous flank H358 tumors will be dosed with EGFR antibody-siRNA-EEP conjugates at 0.5 mg/kg (based on siRNA). Multiple EEPs (endosomolytic moieties) will be used to determine the peptide sequence that demonstrates optimal endosomal escape, resulting in the best knockdown of the target gene relative to the control.

Example 9. Formulation of an ABC conjugate with Nanoparticles

[0524] An exemplary ABC conjugate is packaged into self-assembled nanoparticles using cyclodextrin polymers (10 kDa) and an excess of non-conjugated siRNAs (ED 40-60 nm, PDI 0.1-0.2). In these particles, the exemplary ABC conjugate maintains its ability to interact with the antibody target. The stability and target binding competency of the particles in circulation in vivo is regulated through modifications of the packaging siRNAs.

[0525] **Nanoparticle Formation**

[0526] Nanoparticles are prepared at a final siRNA concentration of 1.6 mg/mL. siRNA containing CY5-siRNA at a ratio of 1:20 is first diluted to 2x final concentration in water. Cyclodextrin polymer (CDP) is diluted to 2x final concentration necessary to achieve a nitrogen to phosphorus ratio (N:P) of 3:1 in 10 mM phosphate buffer at neutral pH. CDP is added quickly to siRNA and is further mixed by pipetting. Particles are incubated for at least 15 minutes before dosing or analysis.

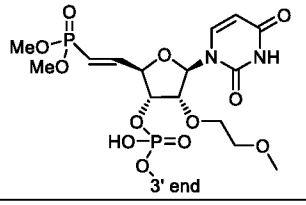
[0527] **In vitro EGFR binding**

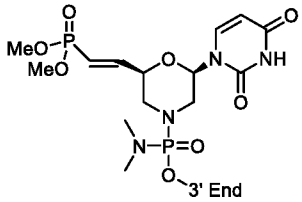
[0528] Nanoparticles containing various amount of the exemplary ABC conjugate are diluted into Fetal calf serum to a final concentration of 10 nM and are incubated for 1h at RT with Protein G Dynabeads (Thermofisher) loaded with 150 nM of a purified EGFR-Fc protein (Sino Biological). Beads are washed twice with PBS containing 0.01% Tween 20 and 0.05% BSA before bead-bound nanoparticles are disrupted with water containing 0.01% Tween 20 and 100 ug/ml heparin. The amount of CY5-siRNA contained in the input, unbound fraction, washes and bead eluate is quantified by fluorescence using a TECAN Infinite M200 Pro (Excitation 635 nm; Emission 675 nm).

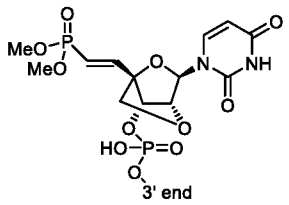
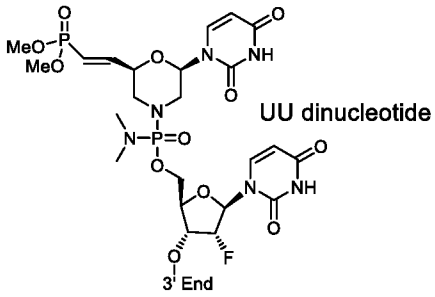
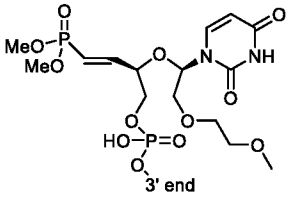
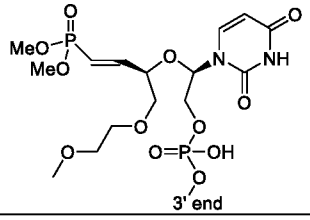
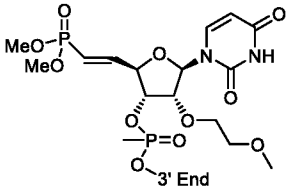
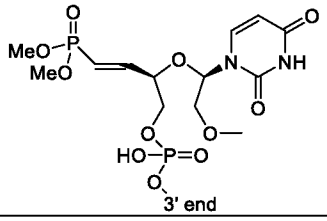
Example 10. siRNA synthesis

[0529] All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified using HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity.

[0530] The vinylphosphonate modified nucleotides (compounds 3, 15, 26, 27 and 28) used for examples 11 - 15 are shown in the table below

Structure	Cmpd #
	3

Structure	Cmpd #
	

	15		28
	26		30
	27		32

[0531] Compound 3 was used as a standard for comparison purposes.

[0532] Compounds 15, 26, 27, 28, 30 and 32 were incorporated onto individual guide strands.

[0533] Compound 28 was incorporated onto the 5' end of the passenger during solid phase synthesis as a dinucleotide.

[0534] All the siRNA passenger strands contained a C6-NH_2 conjugation handle on the 5' end.

[0535] For the 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs, the conjugation handle was connected to siRNA passenger strand via an inverted abasic phosphodiester, (see figure 1A).

[0536] For the blunt ended duplex with 19 bases of complementarity and one 3' dinucleotide overhang the conjugation handle was connected to siRNA passenger strand via a phosphodiester on the terminal base, (see figure 1B).

[0537] Purified single strands were duplexed to obtain double stranded siRNA.

Example 11. *In vitro* activity of vinylphosphonate modified nucleotide structures in HCT116 cells

[0538] **siRNA design and synthesis**: A 21mer HPRT guide strand was designed against mouse HPRT. The sequence (5' to 3') of the guide/antisense strand was UUAAAAUCUACAGUCAUAGUU.

[0539] Three versions were prepared incorporating vinylphosphonate modified nucleotide structures (compounds 3, 15 and 27). The guide and fully complementary 0RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry, and purified

over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to obtain double stranded siRNA as described in figure 1B.

[0540] **In vitro study**: The different siRNAs were tranfected into human colorectal carcinoma HCT116 cells at 100 nM, 10 nM, 1 nM, 0.1 nM, 0.01 nM, 0.001 nM, and 0.0001 nM final concentration. The siRNAs were formulated with commercially available transfection reagent, Lipofectamine RNAiMAX (Life Technologies), according to the manufacturer's "forward transfection" instructions. Cells were plated 24 h prior to transfection in triplicate on 24-well tissue culture plates, with 50000 cells per well. At 48 h post-transfection cells were washed with PBS and harvested with TRIzol® reagent (Life Technologies). RNA was isolated using the Direct-zol-96 RNA Kit (Zymo Research) according to the manufacturer's instructions. 10 µl of RNA was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. cDNA samples were evaluated by qPCR with HPRT-specific and PPIB-specific TaqMan gene expression probes (Thermo Fisher) using TaqMan® Fast Advanced Master Mix (Applied Biosystems). HPRT values were normalized within each sample to PPIB gene expression. The quantification of HPRT downregulation was performed using the standard $2^{-\Delta\Delta Ct}$ method. All experiments were performed in triplicate.

[0541] Figure 2 shows the dose response curves demonstrating that novel vinylphosphonate modified nucleotide structures on the guide strands of an HPRT siRNA, after *in vitro* transfection of the duplex, can be loaded into RISC and mediate sequence specific down regulation of the target HPRT gene. Activity of the analogues (compounds 15 and 27) was comparable to the standard vinylphosphonate modified nucleotide (compound 3).

Example 12. *In vitro* activity of vinylphosphonate modified nucleotide structures in rhabdomyosarcoma cells

[0542] **siRNA design and synthesis**: A 21mer myostatin (MSTN) guide strand was designed against human MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAUUAUUUGUUCUUUGCCUU. Five versions were prepared incorporating different structures (compounds 3, 15, 26, 27 and 28). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry, and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to obtain double stranded siRNA as described in figure 1A.

[0543] **In vitro study**: The activity of the siRNAs was evaluated in transfected human rhabdomyosarcoma cells (SJCRH30, ATCC CRL-2061). Cells were grown in RPMI-1640 supplemented with 10% heat inactivated FBS (Gibco) and 10 mM HEPES and 1 mM sodium pyruvate. For siRNA transfections, cells were plated at a density of 20.000 cells/well on 24 well plates plates and transfected with various concentrations of the siRNAs (0.0001- 100 nM final concentration) using Lipofectamine RNAiMAX (Life Technologies), according to the manufacturer's "forward transfection" instructions. At 72 h post-transfection cells were washed with PBS and harvested with 300 □l/ well TRIzol® reagent (Life Technologies). RNA was isolated using the Direct-zol-96 RNA Kit (Zymo Research) according to the manufacturer's instructions. 10 µl of RNA was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. cDNA samples were evaluated by qPCR with MSTN-specific and PPIB-specific TaqMan gene expression probes (Thermo Fisher) using TaqMan® Fast Advanced Master Mix (Applied Biosystems) and the ΔΔCt method. Individual experiments were performed in duplicate or triplicate.

[0544] The table below shows the the half maximal inhibitory concentrations of the analogs (compounds 15, 26, 27 and 28) and maximium knockdown achieved relative to the standard vinylphosphonate modified nucleotide (compound 3).

Compound on 5' end of GS	**3**	**28**	**26**	**15**	**27**
IC50 (pM)	74.0	55.7	86.5	80.3	16.2
Max KD (% untreated control)	87.77	86.51	84.61	85.47	87.24

[0545] Figure 3 shows the dose response curves, demonstrating that novel vinylphosphonate modified nucleotide structures on the guide strands of an MSTN siRNA, after *in vitro* transfection of the duplex, can be loaded into RISC and mediate sequence specific down regulation of the target MSTN gene. Activity of the analogs (compounds 15, 26, 27 and 28) was comparable to the standard vinylphosphonate modified nucleotide (compound 3).

Example 13. 2017-PK-407-WT: *In vivo* transferrin mAb conjugate delivery of siRNA

[0546] For groups 1-4, the 21mer HPRT guide strand was designed against mouse HPRT. The sequence (5' to 3') of the guide/antisense strand was UUAAAAUCUACAGUCAUAGUU. The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to obtain double stranded siRNA described above. The guide strand utilized the compound 3 vinylphosphonate

modified nucleotide structure. The passenger strand had a conjugation handle on the 5' end via an inverted abasic phosphodiester linkage, see figure 1A.

[0547] For groups 5-8, the 21mer HPRT guide strand was designed against mouse HPRT. The sequence (5' to 3') of the guide/antisense strand was UUAAAAUCUACAGUCAUAGUU. The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to obtain double stranded siRNA described in figure C. The guide strand utilized the compound 15 vinylphosphonate modified nucleotide structure. The passenger strand had a conjugation handle on the 5' end via the phosphodiester linkage on the terminal base, see figure 1B.

[0548] For groups 9-12, the 21mer HPRT guide strand was designed against mouse HPRT. The sequence (5' to 3') of the guide/antisense strand was UUAAAAUCUACAGUCAUAGUU. The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to obtain double stranded siRNA. The guide strand utilized the compound 27 vinylphosphonate modified nucleotide structure. The passenger strand had a conjugation handle on the 5' end via the phosphodiester linkage on the terminal base, see figure 1B

[0549] For groups 13-16, the 21mer MSTN guide strand was designed against human MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAUUAUUUGUUCUUUGCCUU. The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to obtain double stranded siRNA. The guide strand utilized the compound 28 vinylphosphonate modified nucleotide structure. The passenger strand had a conjugation handle on the 5' end via an inverted abasic phosphodiester linkage, see figure 1A.

[0550] For groups 17-20, the 21mer MSTN guide strand was designed against human MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAUUAUUUGUUCUUUGCCUU. The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to obtain double

stranded siRNA. The guide strand utilized the compound 3 vinylphosphonate modified nucleotide structure. The passenger strand had a conjugation handle on the 5' end via an inverted abasic phosphodiester linkage, see figure 1A

[0551] Antibody siRNA Conjugate Synthesis using bis-maleimide (BisMal) linker

Step 1: Antibody reduction with TCEP

[0552] Antibody was buffer exchanged with 25mM borate buffer (pH 8) with 1mM DTPA and made up to 10mg/ml concentration. To this solution, 4 equivalents of TCEP in the same borate buffer were added and incubated for 2 hours at 37°C. The resultant reaction mixture was combined with a solution of BisMal-siRNA (1.25 equivalents) in pH 6.0 10 mM acetate buffer at RT and kept at 4 ^{0}C overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA. The reaction mixture was treated with 10 EQ of N-ethylmaleimide (in DMSO at 10 mg/mL) to cap any remaining free cysteine residues.

Step 2: Purification

[0553] The crude reaction mixture was purified by AKTA Pure FPLC using anion exchange chromatography (SAX) method-1. Fractions containing DAR1 and DAR2 antibody-siRNA conjugates were isolated, concentrated and buffer exchanged with pH 7.4 PBS.

Anion exchange chromatography (SAX) method-1

1. Column: Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 um
2. Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0; Flow Rate: 6.0 ml/min
3. Gradient:
   a. %A %B Column Volume
   b. 100 0 1
   c. 81 19 0.5
   d. 50 50 13
   e. 40 60 0.5
   f. 0 100 0.5
   g. 100 0 2

Anion exchange chromatography (SAX) method-2

1. Column: Thermo Scientific, ProPac™ SAX-10, Bio LC™, 4 X 250 mm
2. Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl; Flow Rate: 0.75 ml/min
3. Gradient:
   a. Time %A %B
   b. 0.0 90 10
   c. 3.00 90 10
   d. 11.00 40 60
   e. 14.00 40 60
   f. 15.00 20 80
   g. 16.00 90 10
   h. 20.00 90 10

Step-3: Analysis of the purified conjugate

[0554] The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2.

Conjugate	**SAX retention time (min)**	**% purity (by peak area)**
TfR-mAb--compound 3-HPRT DAR 1	9.41	99
TfR-mAb-compound 15-HPRT DAR 1	8.83	99
TfR-mAb-compound 27-HPRT DAR 1	8.54	99
TfR-mAb-compound 28-MSTN DAR 1	8.96	99
TfR-mAb-compound 3-MSTN DAR 1	9.39	99

In vivo study

[0555] The conjugates were assessed for their ability to mediate mRNA downregulation of myostatin (MSTN) and HPRT in skeletal muscle, in an *in vivo* experiment (wild type CD-1 mice). Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs and doses, see table below. After 168 hours, gastrocnemius (gastroc) muscle tissues were harvested and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt).

Group	**Test Article**	**N**	**siRNA Dose (mg/kg)**	**Dose Volume (mL/kg)**	**Harvest Time (h)**
1	TfR1-mAb-compound 3-HPRT DAR1	4	3	**5.0**	168
2	TfR1-mAb-compound 3-HPRT DAR1	4	1	**5.0**	168
3	TfR1-mAb-compound 3-HPRT DAR1	4	0.3	**5.0**	168
4	TfR1-mAb-compound 3-HPRT DAR1	4	0.1	**5.0**	168
5	TfR1-mAb-compound 15-HPRT DAR1	4	3.0	**5.0**	168
6	TfR1-mAb-compound 15-HPRT DAR1	4	1	**5.0**	168
7	TfR1-mAb-compound 15-HPRT DAR1	4	0.3	**5.0**	168

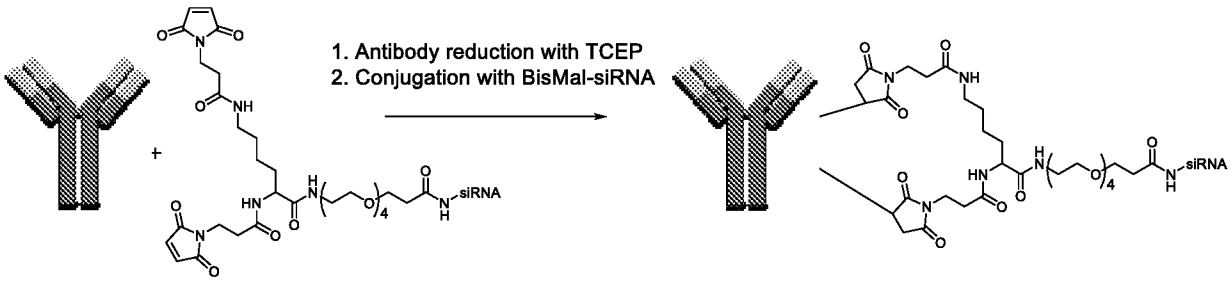
8	TfR1-mAb-compound 15-HPRT DAR1	4	0.1	**5.0**	168
9	TfR1-mAb-compound 27-HPRT DAR1	4	0.5	**5.0**	168
10	TfR1-mAb-compound 27-HPRT DAR1	4	0.5	**5.0**	168
11	TfR1-mAb-compound 27-HPRT DAR1	4	0.5	**5.0**	168
12	TfR1-mAb-compound 27-HPRT DAR1	4	0.5	**5.0**	168
13	TfR1-mAb-compound 28-MSTN DAR1	4	0.5	**5.0**	168
14	TfR1-mAb-compound 28-MSTN DAR1	4	0.5	**5.0**	168
15	TfR1-mAb-compound 28-MSTN DAR1	4	0.5	**5.0**	168
16	TfR1-mAb-compound 28-MSTN DAR1	4	0.5	**5.0**	168
17	TfR1-mAb-compound 3-MSTN DAR1	4	0.5	**5.0**	168
18	TfR1-mAb-compound 3-MSTN DAR1	4	0.5	**5.0**	168
19	TfR1-mAb-compound 3-MSTN DAR1	4	0.5	**5.0**	168
20	TfR1-mAb-compound 3-MSTN DAR1	4	0.5	**5.0**	168
21	PBS control	5		**5.0**	168

[0556] Figure 4 shows that the vinylphosphonate modified nucleotide structures on the siRNA guide strands demonstrated dose dependent downregulation of their target gene in gastric muscle when conjugated to an anti-TfR mAb targeting the transferrin receptor. Activity of the analogues was comparable to the standard vinylphosphonate modified nucleotide structure.

[0557] Example 14. 2017-PK-421-WT: *In vivo* transferrin mAb conjugate delivery of siRNA

[0558] For groups 1-12, the 21mer MSTN guide strand was designed against human/mouse MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAUUAUUUGUUCUUUGCCUU. The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to obtain double stranded siRNA. Three guide strands were produced with three different vinylphosphonate modified nucleotide structures at the 5'end (compounds 3, 15 and 26). The passenger strand had a conjugation handle on the 5' end via a phosphodiester-inverted abasic linkage.

[0559] The anti-TfR1 mAb-MSTN DAR1 conjugates were synthesized as described below:



Step 1: Antibody reduction with TCEP

[0560] Antibody was buffer exchanged with 25mM borate buffer (pH 8) with 1mM DTPA and made up to 10mg/ml concentration. To this solution, 4 equivalents of TCEP in the same borate buffer were added and incubated for 2 hours at 37°C. The resultant reaction mixture was combined with a solution of BisMal-siRNA (1.25 equivalents) in 10 mM acetate buffer (pH 6) at RT and kept at 4 ^{0}C overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA. The reaction mixture was treated with 10 EQ of N-ethylmaleimide (in DMSO at 10 mg/mL) to cap any remaining free cysteine residues.

Step 2: Purification

[0561] The crude reaction mixture was purified by AKTA Pure FPLC using anion exchange chromatography (SAX) method-1. Fractions containing DAR1 and DAR2 antibody-siRNA conjugates were isolated, concentrated and buffer exchanged with pH 7.4 PBS.

Anion exchange chromatography (SAX) method-1.

1. Column: Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 um
2. Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0; Flow Rate: 6.0 ml/min
3. Gradient:

	%A	%B	Column Volume	
a.				
b.	100	0	1	
c.	81		19	0.5
d.	50		50	13
e.	40		60	0.5
f.	0		100	0.5
g.	100	0	2	

Anion exchange chromatography (SAX) method-2

1. Column: Thermo Scientific, ProPac™ SAX-10, Bio LC™, 4 X 250 mm
2. Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl; Flow Rate: 0.75 ml/min
3. Gradient:

	Time	%A	%B	
a.				
b.	0.0		90	10
c.	3.00	90	10	
d.	11.00	40	60	
e.	14.00	40	60	
f.	15.00	20	80	

g. 16.00 90 10
h. 20.00 90 10

Step-3: Analysis of the purified conjugate

The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2.

Conjugate	SAX retention time (min)	% purity (by peak area)
TfR-compound 3-MSTN.mff3s DAR 1	8.99	99
TfR-compound 15-MSTN DAR 1	9.12	99
TfR-compound 26-MSTN DAR 1	9.09	99

In vivo study

[0562] The conjugates were assessed for their ability to mediate mRNA downregulation of myostatin (MSTN) in skeletal muscle, in an *in vivo* experiment (wild type CD-1 mice). Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs and doses, as noted in the table below. After 168 hours, gastrocnemius (gastroc), quadriceps (quad) and heart muscle tissues were harvested and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt).

Group	Test Article	N	siRNA Dose (mg/kg)	Dose Volume (mL/kg)	Harvest Time (h)
1	TfR1-mAb-compound 26-MSTN DAR1	4	3	**5.0**	168
2	TfR1-mAb-compound 26-MSTN DAR1	4	1	**5.0**	168
3	TfR1-mAb-compound 26-MSTN DAR1	4	0.3	**5.0**	168
4	TfR1-mAb-compound 26-MSTN DAR1	4	0.1	**5.0**	168
5	TfR1-mAb-compound 15-MSTN DAR1	4	3.0	**5.0**	168
6	TfR1-mAb-compound 15-MSTN DAR1	4	1	**5.0**	168

7	TfR1-mAb-compound 15-MSTN DAR1	4	0.3	**5.0**	168
8	TfR1-mAb-compound 15-MSTN DAR1	4	0.1	**5.0**	168
9	TfR1-mAb-compound 3-MSTN DAR1	4	0.5	**5.0**	168
10	TfR1-mAb-compound 3-MSTN DAR1	4	0.5	**5.0**	168
11	TfR1-mAb-compound 3-MSTN DAR1	4	0.5	**5.0**	168
12	TfR1-mAb-compound 3-MSTN DAR1	4	0.5	**5.0**	168
13	PBS control	5		**5.0**	168

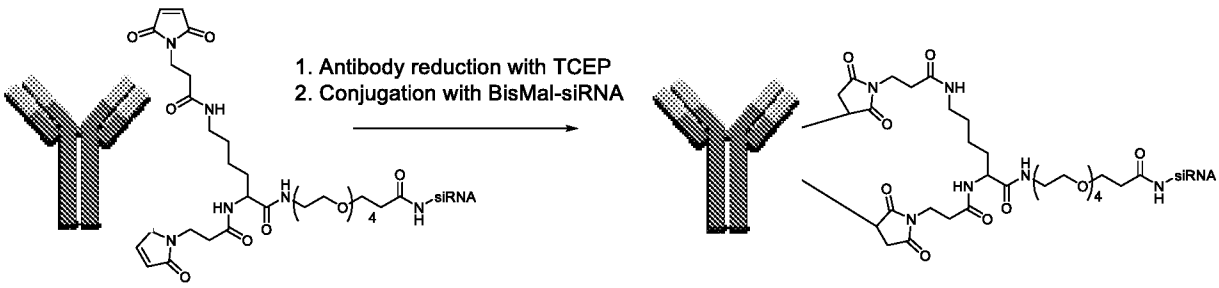
[0563] Figure 5 demonstrates MSTN mRNA downregulation in gastroc, quad and heart muscle with antibody siRNA conjugates containing different vinylphosphonate modified nucleotide structure on the 5' end of the guide strand. Activity of the analogues was comparable to the standard vinylphosphonate modified nucleotide structure.

[0564] Example 15. 2017-PK-422-WT: In vivo transferrin mAb conjugate delivery of siRNA

[0565] For groups 1-4 and 9-12, the 21mer HPRT guide strand was designed against mouse HPRT. The sequence (5' to 3') of the guide/antisense strand was UUAAAAUCUACAGUCAUAGUU. The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to obtain double stranded siRNA. The guide strand was produced with compound 3 vinylphosphonate modified nucleotide structures at the 5'end. The passenger strand had a conjugation handle on the 5' end via aphosphorothioate-inverted abasic-phosphodiester linker, see figure 1A.

[0566] For groups 5-8 and 13-16 a 21mer HPRT guide strand was designed against mouse HPRT. The sequence (5' to 3') of the guide/antisense strand was UUAAAAUCUACAGUCAUAGUU. The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to obtain double stranded siRNA. The guide strand was produced with a compound 26 vinylphosphonate modified nucleotide structures at the 5'end. The passenger strand had a conjugation handle on the 5' end via the terminal phosphorothioate., see figure 1B.

[0567] The anti-TfR1 mAb-HPRT DAR1 conjugates were synthesized as described below:



Step 1: Antibody reduction with TCEP

[0568] Antibody was buffer exchanged with 25mM borate buffer (pH 8) with 1mM DTPA and made up to 10mg/ml concentration. To this solution, 4 equivalents of TCEP in the same borate buffer were added and incubated for 2 hours at 37°C. The resultant reaction mixture was combined with a solution of BisMal-siRNA (1.25 equivalents) in 10 mM acetate buffer (pH 6) at RT and kept at 4 ^{0}C overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA. The reaction mixture was treated with 10 EQ of N-ethylmaleimide (in DMSO at 10 mg/mL) to cap any remaining free cysteine residues.

Step 2: Purification

[0569] The crude reaction mixture was purified by AKTA Pure FPLC using anion exchange chromatography (SAX) method-1. Fractions containing DAR1 and DAR2 antibody-siRNA conjugates were isolated, concentrated and buffer exchanged with pH 7.4 PBS.

Anion exchange chromatography (SAX) method-1.

1. Column: Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 um
2. Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0; Flow Rate: 6.0 ml/min
3. Gradient:

	%A	%B	Column Volume
a.			
b.	100	0	1
c.	81	19	0.5
d.	50	50	13
e.	40	60	0.5
f.	0	100	0.5
g.	100	0	2

Anion exchange chromatography (SAX) method-2

1. Column: Thermo Scientific, ProPac™ SAX-10, Bio LC™, 4 X 250 mm
2. Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl; Flow Rate: 0.75 ml/min
3. Gradient:

	Time	%A	%B
a.			
b.	0.0	90	10
c.	3.00	90	10
d.	11.00	40	60
e.	14.00	40	60

f. 15.00 20 80
g. 16.00 90 10
h. 20.00 90 10

Step-3: Analysis of the purified conjugate

[0570] The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2.

Conjugate	SAX retention time (min)	% purity (by peak area)
TfR-compound 3-HPRT DAR 1	9.14	99
TfR-compound 26-HPRT DAR 1	8.54	99
ASGR-compound 3-HPRT DAR 1	9.3	99
ASGR-compound 26-MSTN DAR 1	8.62	99

In vivo study

[0571] The conjugates were assessed for their ability to mediate mRNA downregulation of HPRT in muscle and liver, in an *in vivo* experiment (wild type CD-1 mice). Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs and doses, as noted in the table below. After 168 hours, gastrocnemius (gastroc), and liver tissues were harvested and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt).

Group	Test Article	N	siRNA Dose (mg/kg)	Dose Volume (mL/kg)	Harvest Time (h)
1	TfR1-mAb-compound 3-HPRT DAR1	4	1	5.0	168
2	TfR1-mAb-compound 3-HPRT DAR1	4	0.3	5.0	168
3	TfR1-mAb-compound 3-HPRT DAR1	4	0.1	5.0	168
4	TfR1-mAb-compound 3-HPRT DAR1	4	0.03	5.0	168
5	TfR1-mAb-compound 26-HPRT DAR1	4	1	5.0	168
6	TfR1-mAb-compound 26-HPRT DAR1	4	0.3	5.0	168

7	TfR1-mAb-compound 26-HPRT DAR1	4	0.1	5.0	168
8	TfR1-mAb-compound 26-HPRT DAR1	4	0.03	5.0	168
9	ASGR-mAb-compound 3-HPRT DAR1	4	1	5.0	168
10	ASGR-mAb-compound 3-HPRT DAR1	4	0.3	5.0	168
11	ASGR-mAb-compound 3-HPRT DAR1	4	0.1	5.0	168
12	ASGR-mAb-compound 3-HPRT DAR1	4	0.03	5.0	168
13	ASGR-mAb-compound 26-HPRT DAR1	4	1	5.0	168
14	ASGR-mAb-compound 26-HPRT DAR1	4	0.3	5.0	168
15	ASGR-mAb-compound 26-HPRT DAR1	4	0.1	5.0	168
16	ASGR-mAb-compound 26-HPRT DAR1	4	0.03	5.0	168
17	PBS control	5		5.0	168

[0572] Figure 6 demonstrates the modified vinylphosphonate modified nucleotide structures on the siRNA guide strands were able to mediate dose dependent downregulation of the HPRT target gene in the mouse liver and gastroc muscle after IV administration to a mouse. When conjugated to an anti-TfR mAb targeting the transferrin receptor on muscle, the siRNA was able to mediate HPRT downregulation in muscle. When conjugated to an anti-ASGR mAb targeting the ASGR receptor on liver hepatocytes, the siRNA was able to mediate HPRT downregulation in liver.

[0573] Example 16. In vitro activity of vinylphosphonate modified nucleotide structures in HCT116 cells

[0574] siRNA design and synthesis: A 21mer SSB guide strand was designed against mouse SSB. The sequence (5' to 3') of the guide/antisense strand was UUACAUUAAAGUCUGUUGUUU. Three versions were made incorporating vinylphosphonate modified nucleotide structures (compounds 3, 30 and 32). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry, and purified over HPLC. The vinylphosphonate modified nucleotide structures were incorporated using the amidites described in figure A3. The Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described above.

[0575] In vitro study: The different siRNAs were transfected into human colorectal carcinoma HCT116 cells at 100 nM, 10 nM, 1 nM, 0.1 nM, 0.01 nM, 0.001 nM, and 0.0001 nM final concentration. The siRNAs were formulated with commercially available transfection reagent.\, Lipofectamine RNAiMAX (Life Technologies), according to the manufacturer's "forward

transfection" instructions. Cells were plated 24 h prior to transfection in triplicate on 24-well tissue culture plates, with 50000 cells per well. At 48 h post-transfection cells were washed with PBS and harvested with TRIzol® reagent (Life Technologies). RNA was isolated using the Direct-zol-96 RNA Kit (Zymo Research) according to the manufacturer's instructions. 10 µl of RNA was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. cDNA samples were evaluated by qPCR with HPRT-specific and PPIB-specific TaqMan gene expression probes (Thermo Fisher) using TaqMan® Fast Advanced Master Mix (Applied Biosystems). HPRT values were normalized within each sample to PPIB gene expression. The quantification of HPRT downregulation was performed using the standard $2^{-\Delta\Delta Ct}$ method. All experiments were performed in triplicate.

[0576] EC_{50} values were as follows:

Sample	**EC50 (pM)**
Compound 3-SSB	4.00
Compound 30-SSB	0.44
Compound 32-SSB	1.46

[0577] Figure 7 shows the dose response curves demonstrating that novel vinylphosphonate modified nucleotide structures on the 5' end of the guide strands of an SSB siRNA, after *in vitro* transfection of the duplex, can be loaded into RISC and mediate sequence specific down regulation of the target SSB gene. Activity of the analogues (compounds 30 and 32) was comparable to the standard vinylphosphonate modified nucleotide structure (compound 3).

[0578] Example 17. *In vitro* activity vinylphosphonate modified nucleotide structures in rhabdomyosarcoma cells

[0579] siRNA design and synthesis: A 21mer myostatin (MSTN) guide strand was designed against mouse and human MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAUUAUUUGUUCUUUGCCUU. Four versions were prepared incorporating different structures (compounds 3, 26, 30 and 32). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry, and purified using HPLC. The vinylphosphonate modified nucleotide structures were incorporated using the amidites as described above. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA as described above.

[0580] In addition, a 21mer SSB guide strand was designed against mouse SSB. The sequence (5' to 3') of the guide/antisense strand was UUACAUUAAAGUCUGUUGUUU. Three

versions were made with different vinylphosphonate modified nucleotide structures (compounds 3, 30 and 32). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry, and purified over HPLC. The vinylphosphonate modified nucleotide structures were incorporated using the amidites as described herein. The Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA as described above.

[0581] In vitro study: The activity of the siRNAs was evaluated in transfected human rhabdomyosarcoma cells (SJCRH30, ATCC CRL-2061). Cells were grown in RPMI-1640 supplemented with 10% heat inactivated FBS (Gibco) and 10 mM HEPES and 1 mM sodium pyruvate. For siRNA transfections, cells were plated at a density of 20.000 cells/well on 24 well plates plates and transfected with various concentrations of the siRNAs (0.0001- 100 nM final concentration) using Lipofectamine RNAiMAX (Life Technologies), according to the manufacturer's "forward transfection" instructions. At 72 h post-transfection cells were washed with PBS and harvested with 300 ul/ well TRIzol® reagent (Life Technologies). RNA was isolated using the Direct-zol-96 RNA Kit (Zymo Research) according to the manufacturer's instructions. 10 µl of RNA was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. cDNA samples were evaluated by qPCR with MSTN-specific and PPIB-specific TaqMan gene expression probes (Thermo Fisher) using TaqMan® Fast Advanced Master Mix (Applied Biosystems) and the ΔΔCt method. Individual experiments were performed in duplicate or triplicate.

[0582] Figure 8A shows the dose response curves, demonstrating that the novel vinylphosphonate modified nucleotide structures on the guide strands of an MSTN siRNA, after *in vitro* transfection of the duplex, can be loaded into RISC and mediate sequence specific down regulation of the target MSTN gene. Activity of the analogues (compounds 26, 30 and 32) was comparable to the standard vinylphosphonate modified nucleotide (compound 3).

[0583] Figure 8B shows the dose response curves, demonstrating that the novel vinylphosphonate modified nucleotide structures on the guide strands of an SSB siRNA, after *in vitro* transfection of the duplex, can be loaded into RISC and mediate sequence specific down regulation of the target SSB gene. Activity of the analogues (30 and 32) was comparable to a standard vinylphosphonate modified nucleotide (compound 3).

[0584] Example 18. *In vitro* activity of vinyl phosphonate modified nucleotide structures in apparently healthy human-derived immortalized skeletal muscle myoblast cells (MB)

[0585] siRNA design and synthesis: A 21mer myostatin (MSTN) guide strand was designed against mouse and human MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAUUAUUUGUUCUUUGCCUU. Four versions were prepared incorporating different vinylphosphonate modified nucleotide structures (compounds 3, 26, 30 and 32). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry, and purified using HPLC. The vinylphosphonate modified nucleotide structures were incorporated using the amidites described herein. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA as described above.

[0586] In addition, a 21mer SSB guide strand was designed against mouse SSB. The sequence (5' to 3') of the guide/antisense strand was UUACAUUAAAGUCUGUUGUUU. Three versions were prepared incorporating different vinylphosphonate modified nucleotide structures (compounds 3, 30 and 32). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phospharamidite chemistry, and purified over HPLC. The vinylphosphonate modified nucleotide structures were incorporated using the amidites described herein. The Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA as described above.

[0587] In vitro study: The activity of the siRNAs was evaluated in apparently healthy human-derived immortalized skeletal muscle myoblasts (MB) (obtained from Denis Furling, Institut de Myologie, France). MB cells were grown in a complete skeletal muscle cell growth medium (PromoCell). Cells were plated 24 h prior to transfection in triplicate on 96-well tissue culture plates, with 4000 (MB) cells per well. siRNAs were transfected into cells 50 nM, 5.5556 nM, 0.6173 nM, 0.0686 nM, 0.0076 nM, 0.0008 nM, and 0.0001 nM final concentration. The siRNAs were formulated with commercially available transfection reagent Lipofectamine RNAiMAX (Life Technologies), according to the manufacturer's "forward transfection" protocol instructions. At 48 h post-transfection cells were washed with PBS and harvested with TRIzol® reagent (Life Technologies). RNA was isolated using the Direct-zol-96 RNA Kit (Zymo Research) according to the manufacturer's instructions. 10 µl of RNA was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. cDNA samples were evaluated by qPCR with SSB-specific, MSTN-specific and PPIB-specific TaqMan gene expression probes (Thermo Fisher) using TaqMan® Fast Advanced Master Mix (Applied Biosystems). SSB and MSTN expression values were normalized within each sample to PPIB gene expression. The

quantification of SSB and MSTN downregulation was performed using the standard 2-ΔΔCt method. All experiments were performed in triplicate.

[0588] Figure 9A shows the dose response curves, demonstrating that the novel vinylphosphonate modified nucleotide structures on the guide strands of an MSTN siRNA, after *in vitro* transfection of the duplex, can be loaded into RISC and mediate sequence specific down regulation of the target MSTN gene. Activity of the analogues (compounds 26, 30 and 32) was comparable to the standard vinylphosphonate modified nucleotide (compound 3).

[0589] Figure 9B shows the dose response curves, demonstrating that the novel vinylphosphonate modified nucleotide structures on the guide strands of an SSB siRNA, after *in vitro* transfection of the duplex, can be loaded into RISC and mediate sequence specific down regulation of the target SSB gene. Activity of the analogues (30 and 32) was comparable to a standard vinylphosphonate modified nucleotide (compound 3).

[0590] 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.

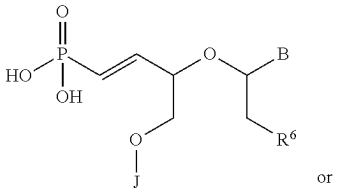
CLAIMS

What is claimed is:

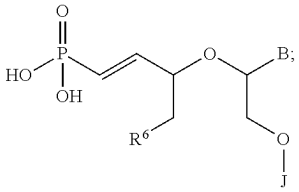
1. A polynucleotide conjugate molecule comprising a binding moiety and a polynucleotide,

wherein the polynucleotide comprises at least one 5′-vinylphosphonate modified non-natural nucleotide and at least one modified internucleotide linkage, or at least one inverted abasic moiety; and

wherein the at least one 5′-vinylphosphonate modified non-natural nucleotide is:



or



wherein B is a heterocyclic base moiety;

R^6 is selected from hydrogen, halogen, alkyl, alkoxy or aminoalkyl; and

J is an internucleotide linking group linking to an adjacent nucleotide of the polynucleotide.

2. The molecule of claim 1, wherein the at least one 5′-vinylphosphonate modified non-natural nucleotide is located at the 5′-terminus of the polynucleotide.

3. The molecule of claim 1, wherein the at least one 5′-vinylphosphonate modified non-natural nucleotide is located at an internucleotide linkage of the polynucleotide.

4. The molecule of claim 1, wherein the at least one 5′-vinylphosphonate modified non-natural nucleotide is further modified at the 2′-position.

5. The molecule of claim 4, wherein the 2′-modification is 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-dimethylaminoethyloxyethyl(2′-O-DMAEOE), 2′-O—N-methylacetamido (2′-O-NMA), 2′-O-ethyoxyethyl(2′-O-EOE), 2′-O-(2-N-Methylcarbamoylethyl), PEG1, or PEG2 modified

nucleotide.

6. The molecule of claim 1, wherein the at least one modified internucleotide linkage comprises a phosphorothioate linkage, a phosphorodithioate linkage, a phosphorodiamidate linkage, a methylphosphonate linkage, or an amide linkage.

7. The molecule of claim 1, wherein the at least one inverted abasic moiety is at least one terminus.

8. The molecule of claim 1, wherein the polynucleotide comprises a single-stranded polynucleic acid molecule.

9. The molecule of claim 1, wherein the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule.

10. The molecule of claim 9, wherein the second polynucleotide comprises at least one modification.

11. The molecule of claim 9, wherein the first polynucleotide and the second polynucleotide are RNA molecules.

12. The molecule of claim 1, wherein the polynucleotide is coupled to the binding moiety via a bond.

13. The molecule of claim 1, wherein the polynucleotide is coupled to the binding moiety via a C_1-C_6 alkyl group.

14. The molecule of claim 1, wherein the polynucleotide is coupled to the binding moiety via X is a homobifunctional linker or a heterobifunctional linker, optionally conjugated to a C_1-C_6 alkyl group.

15. The molecule of claim 1, further comprising a polymer.

16. The molecule of claim 1, wherein the binding moiety comprises a humanized antibody or antigen binding fragment thereof, a chimeric antibody or antigen binding fragment thereof, a monoclonal antibody or antigen 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 antigen binding fragment thereof.

17. The molecule of claim 1, wherein the binding moiety comprises a peptide or a small molecule.

18. The molecule of claim 15, wherein the polymer is polyethylene glycol.

19. The molecule of claim 15, wherein the polymer has a molecular weight of about 1000 Da, 2000 Da, or 5000 Da.

20. The molecule of claim 1, further comprising endosomolytic moiety.