

CAPPED MRNA AND METHOD OF PREPARATION THEREOF**FIELD**

[0001] The present disclosure relates in some aspects to methods to produce modified RNA molecules with enhanced stability.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The contents of the electronic sequence listing (P2024TC2948.xml; Size: 133,721 bytes; and Date of Creation: July 23, 2024) are herein incorporated by reference in their entirety.

BACKGROUND

[0003] RNA molecules produced by *in vitro* transcription offer the potential for valuable and much needed pharmaceutical compositions by allowing the delivery of genetic material to patient cells capable of being translated into proteins of interest. In some aspects, existing methods are limited by the immunostimulation of *in vitro* transcribed RNA molecules, resulting in inefficient expression of the protein of interest within transfected cells. Provided herein are methods and compositions that address such and other needs.

SUMMARY

[0004] In some aspects, provided herein is a modified RNA molecule, wherein the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, and wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine in the molecule.

[0005] In some embodiments, the 5' end of the modified RNA molecule has the sequence of N1pppN2pU or N1vpppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine.

[0006] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of N1pppN2pU or N1vpppN2pU, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine, and wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine.

[0007] In some embodiments, at least about 50% of the uridines other than the first 5' uridine in the molecule are modified. In some embodiments, all of the uridines in the molecule are modified except for the first 5' uridine.

[0008] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of N1pppN2pU or N1vpppN2pU, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine, and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0009] In some embodiments, the 5' end of the modified RNA molecule has the sequence of GpppApU or GvpppApU, wherein A is modified or unmodified. In some embodiments, the 5' end of the modified RNA molecule has the sequence of m⁷GpppApU or m⁷GvpppApU. In some embodiments, the A is modified, preferably 2'-O-methylated.

[0010] In some embodiments, at least about 50% of the uridines other than the first 5' uridine in the molecule are m1Ψ (1-methyl-Pseudouridine). In some embodiments, all of the uridines in the molecule are m1Ψ except for the first 5' uridine.

[0011] In some embodiments, the modified RNA molecule comprises an open reading frame comprising a coding sequence.

[0012] In some embodiments, the coding sequence encodes a therapeutic payload, which comprises a compound capable of eliciting immunity against one or more target conditions or diseases. In some embodiments, the target condition is related to or caused by infection by a pathogen, such as a coronavirus (e.g., 2019-nCoV), influenza, measles, human papillomavirus (HPV), rabies, meningitis, whooping cough, tetanus, plague, hepatitis, and tuberculosis. In some embodiments, the coding sequence encodes a pathogenic protein characteristic for the pathogen, or an antigenic fragment or epitope thereof. In some embodiments, the target condition is related to or caused by neoplastic growth of cells, such as a cancer. In some embodiments, the coding sequence encodes a tumor-associated antigen (TAA) characteristic for the cancer, or an antigenic fragment or epitope thereof.

[0013] In some embodiments, the coding sequence encodes eGFP. In some embodiments, the coding sequence encodes SARS-COV-2 BA.4/BA.5 RBD. In some embodiments, the coding sequence encodes a rabies virus antigen. In some embodiments, the coding sequence encodes a respiratory syncytial virus antigen. In some embodiments, the coding sequence encodes a varicella-zoster virus antigen.

[0014] In some embodiments, the molecule further comprises at least one modified G, C, or A.

[0015] In some embodiments, the molecule has a modified backbone. In some embodiments, the modified backbone comprising at least one phosphorothioate linkage.

[0016] In other aspects, provided herein is a pharmaceutical composition comprising the modified RNA disclosed herein, further comprising a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutically acceptable carrier is an LNP. In some embodiments, the LNP comprises a cationic lipid.

[0017] In some embodiments, the LNP comprises: i) between about 30 to 55 mol percent of a cationic lipid; ii) between about 5 to 40 mol percent of a phospholipid; iii) between about 20 to 50 mol percent of a sterol; and iv) a polymer conjugated lipid.

[0018] In other aspects, provided herein is a method of *in vitro* transcribing a DNA molecule to a modified RNA molecule, comprising combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, wherein the 5' cap has the sequence of N1pppN2pU or N1vpppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine.

[0019] In some embodiments, the *in vitro* transcription mix comprises modified uridines.

[0020] In some embodiments, at least one uridine in the modified RNA molecule is a modified uridine except for the first 5' uridine.

[0021] In some embodiments, the method comprises combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, wherein the 5' cap has the sequence of N1pppN2pU or N1vpppN2pU, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine, wherein the *in vitro* transcription mix comprises modified uridines, and wherein at least one uridine in the modified RNA molecule is a modified uridine except for the first 5' uridine.

[0022] In some embodiments, all of the uridines in the molecule are modified except for the first 5' uridine.

[0023] In some embodiments, the method comprises combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase,

modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, wherein the 5' cap has the sequence of N1pppN2pU or N1vpppN2pU, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine, wherein the in vitro transcription mix comprises modified uridines, and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0024] In some embodiments, the modified uridines are m1Ψ. In some embodiments, all of the uridines in the modified RNA molecule are m1Ψ except for the first 5' uridine.

[0025] In some embodiments, the 5' cap has the sequence of GpppApU or GvpppApU, wherein A is modified or unmodified. In some embodiments, the 5' cap has the sequence of m⁷GpppApU or m⁷GvpppApU. In some embodiments, the A is modified, preferably 2'-O-methylated. In some embodiments, the 5' cap has the sequence of m⁷GpppA_mpU or m⁷GvpppA_mpU.

[0026] In some embodiments, the DNA-dependent RNA polymerase is a T7 RNA polymerase or the variant thereof.

[0027] In some embodiments, the method further comprises isolating the modified RNA molecule.

[0028] In other aspects, provided herein is a modified RNA molecule produced by the method disclosed herein.

[0029] In other aspects, provided herein is a kit comprising: 1) a DNA-dependent RNA polymerase; 2) modified and/or unmodified nucleotides, and 3) a 5' cap, and 4) an instruction for carrying out the method disclosed herein, wherein the 5' cap has the sequence of N1pppN2pU or N1vpppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine.

[0030] In other aspects, provided herein is a method of lowering the innate immune stimulation of a RNA molecule, comprising using a 5' cap having the sequence of N1pppN2pU or N1vpppN2pU and modifying at least one uridine in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine.

[0031] In some embodiments, the 5' cap has the sequence of GpppApU or GvpppApU, wherein A is modified or unmodified. In some embodiments, the 5' cap has the sequence

of $m^7GpppApU$ or $m^7GvpppApU$. In some embodiments, the 5' cap has the sequence of $m^7GpppA_{mp}U$ or $m^7GvpppA_{mp}U$. In some embodiments, the method comprises modifying all of the uridines in the RNA molecule except for the first 5' uridine. In some embodiments, the method comprises modifying all of the uridines in the RNA molecule to $m^1\Psi$ except for the first 5' uridine.

BRIEF DESCRIPTION OF DRAWINGS

[0032] FIG. 1 shows the expression of mRNAs encoding eGFP protein synthesized with $(G^{m7})ppp(A^{m2})G$ and $(G^{m7})ppp(A^{m2})U$ respectively in BHK-21 cell-line.

[0033] FIG. 2 shows in vitro expression of mRNA synthesized by $(G^{m7})ppp(A^{m2})U$ and $(G^{m7})vppp(A^{m2})U$ in HEK-293T cells.

[0034] FIG. 3 shows in vitro expression of mRNA synthesized by $(G^{m7})ppp(A^{m2})U$ and $(G^{m7})vppp(A^{m2})U$ in A549 cells.

DETAILED DESCRIPTION

[0035] All publications, comprising patent documents, scientific articles and databases, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

[0036] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

I. OVERVIEW

[0037] Provided herein in some aspects are modified RNA molecules, wherein the modified RNA molecules comprise a 5' cap, and wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine in the molecule. In some embodiments, the modified RNA molecules are not self-replicable.

[0038] Also provided are methods of *in vitro* transcribing a DNA molecule to a modified RNA molecule, comprising combining the DNA molecule with an *in vitro* transcription mix comprising a DNA dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, and wherein the 5' cap has the sequence of $N1pppN2pU$ or $N1vpppN2pU$,

wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine. Also provided are compositions, formulations, and kits for use in accordance with the provided methods.

[0039] Currently, RNA molecules produced via *in vitro* transcription and transfected into cells often stimulate innate immune receptors such as endosomal and cytosolic pattern recognition receptors (PRRs) including toll-like receptors 3, 7, and 8 (Ma, X., and Hur, S. *Drug Metabolism Pharmacokinet.* 2022. 44, 100450). These toll-like receptors sense single and double stranded RNA and generate immune responses through intracellular signaling cascades. Activation of immune responses in cells transfected with *in vitro* transcribed RNA molecules is a significant limitation to current gene therapy approaches.

[0040] The invention of the present application provides novel methods of *in vitro* mRNA synthesis using a specific uridine-containing cap, for example, m⁷GpppA_mpU cap, m⁷GvpppA_mpU cap, and mRNAs produced by such methods. m⁷GpppA_mpU cap was traditionally used for self-replicating RNAs based on their functions in positive sense strand RNA viruses such as Venezuelan equine encephalitis virus (VEEV), Semliki forest virus (SFV), and Sindbis virus (SIN). The inventors have for the first time used such cap structure for *in vitro* transcription of non-replicable mRNA. It was surprisingly discovered that, compared to mRNAs synthesis methods using a m⁷GpppA_mpG cap, one-step synthesis using a m⁷GpppA_mpU cap allows higher capping rate. The non-replicable mRNAs produced thereby, which may be fully uridine modified except for the first uridine, led to lower innate immune stimulation when delivered to a cell.

[0041] Accordingly, the present invention in one aspect provides a method of *in vitro* transcribing a DNA molecule to a modified RNA molecule, comprising combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, wherein the 5' cap has the sequence of m⁷GpppA_mpU or m⁷GvpppA_mpU, wherein U is an unmodified uridine, wherein the *in vitro* transcription mix comprises modified uridines and wherein all of the uridines in the modified RNA molecule are modified except for the first 5' uridine.

[0042] In another aspect, there is provided a modified RNA molecule, wherein the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, and wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine in the molecule.

[0043] Also provided are kits for carrying out the methods described herein and compositions (such as pharmaceutical compositions) comprising the modified RNA molecules.

[0044] In other aspects, provided herein is a method of lowering the innate immune stimulation of a RNA molecule, comprising using a 5' cap having the sequence of N1pppN2pU or N1vpppN2pU and modifying at least one uridine in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine.

II. DEFINITIONS

[0045] For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth below conflicts with any document incorporated herein by reference, the definition set forth shall control.

[0046] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0047] Throughout this disclosure, various aspects of the claimed subject matter are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the claimed subject matter. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the claimed subject matter. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the claimed subject matter, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the claimed subject matter. This applies regardless of the breadth of the range.

[0048] As used herein, “cap” refers to a non-extendible trinucleotide that facilitates translation or localization, and/or prevents degradation of an RNA transcript when incorporated at the 5' end of an RNA transcript.

[0049] As used herein, the term “nucleobase” refers to a nitrogen containing heterocyclic moiety nucleobase. Non-limiting examples of suitable nucleobases include: adenine, cytosine, guanine, thymine, uracil, or analogs thereof, e.g., 5-propynyl-uracil, 2-thio-5-propynyl-uracil, 5-methylcytosine, pseudoisocytosine, 2-thiouracil, 2-thiothymine, 2-aminopurine, N9-(2-amino-6-chloropurine), N9-(2,6-diaminopurine), hypoxanthine, N9-(7-deaza-guanine), N9-(7-deaza-8-aza-guanine) and N8-(8-aza-7-deazaadenine).

[0050] As used herein, the terms, “ribonucleotide” or “nucleotide” refer to a compound consisting of a nucleobase linked to the C- carbon of a ribose sugar or analog thereof. The ribose or analog may be substituted or unsubstituted.

[0051] As used herein, a “nucleotide triphosphate” refers to a nucleotide with a triphosphate ester group at the 5' position.

[0052] As used here, the terms, “polynucleotide”, “oligonucleotide”, and “nucleic acid” refer to single stranded or double stranded polymers of nucleotide monomers, including ribonucleotides (RNA) and 2'-deoxyribonucleitdes (DNA) linked by internucleotide phosphodiester bond linkages. A polynucleotide may be composed entirely of deoxyribonucleotides, entirely of ribonucleotides, or a chimeric mixture thereof.

[0053] As used herein, a “self-replicable” RNA refers to an RNA molecule that, when delivered to a vertebrate cell, leads to the production of multiple daughter RNAs by transcription from itself via an antisense copy which it generates from itself. A self-replicating RNA molecule is typically a +-strand molecule which can be directly translated after delivery to a cell, thereby providing an RNA-dependent RNA polymerase a template to produce both antisense and sense transcripts from the delivered RNA.

[0054] As used herein, by “pharmaceutically acceptable” or “pharmacologically compatible” is meant a material that is not biologically or otherwise undesirable, e.g., the material may be incorporated into a pharmaceutical composition administered to a patient without causing any significant undesirable biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained. Pharmaceutically acceptable carriers or excipients have preferably met the required standards of toxicological and manufacturing testing and/or are included on the Inactive Ingredient Guide prepared by the U.S. Food and Drug administration.

[0055] As used herein, “a pharmaceutically acceptable carrier” refers to a pharmaceutically acceptable substrate, composition or vehicle used in the process of drug delivery, which may have one or more ingredients including, but not limited to, excipient(s), binder(s), diluent(s), solvent(s), filler(s), and/or stabilizer(s).

[0056] As used herein, the term “lipid” refers to a group of compounds including, without limitation, fats, sterols, waxes, fat-soluble vitamins, monoglycerides, diglycerides, sphingolipids, and phospholipids. In the context of the present disclosure, phospholipids, ionizable lipids, polymer conjugated lipids, and lipid stabilizers are considered lipids.

[0057] As used herein, the term “ionizable lipid” refers to a lipid that has a non-zero net electric charge at physiological pH. The term is inclusive with respect to cationic lipids, including lipids that have a partial positive charge at physiological pH. The term is also inclusive with respect to mixtures of ionizable lipids, which could contain two or more ionizable lipids. In every case where an embodiment is contemplated with the term “ionizable lipid,” it is likewise contemplated with a “cationic lipid,” as though all embodiments were specifically and individually listed with both ionizable and cationic lipids.

[0058] As used herein, the term “polymer conjugated lipid” refers to a lipid comprising a polymer moiety. The term is inclusive with respect to PEGylated lipids, including PEGylated phosphatidylethanolamines, PEGylated phosphatidic acids, PEGylated ceramides, PEGylated dialkylamines, PEGylated diacylglycerols, and PEGylated dialkylglycerols. The term is also inclusive with respect to mixture of polymer conjugated lipids, which may contain two or more polymer conjugated lipids. In every case where an embodiment is contemplated with the term “polymer conjugated lipid,” it is likewise contemplated with a “PEGylated lipid,” as though all embodiments were specifically and individually listed with both polymer conjugated and PEGylated lipids.

[0059] As used herein, the term “lipid stabilizer” refers to a component of the lipid nanoparticle that thought to help stabilize the LNP structure. Without being bound by theory, it is believed that the lipid stabilizer component of LNPs helps favor the liquid-ordered phase of the lipid membrane in LNPs. See, for example, section 3.3.1 of Albertsen, H. C.; et al., “The role of lipid components in lipid nanoparticles for vaccines and gene therapy.” *Adv Drug Deliv Rev.* 2022 Sep; 188:114416. Compounds that can serve as lipid stabilizers include sterols, corticosteroids, vitamins, and other compounds comprising a steroid core.

[0060] As used herein, the term “alkyl” refers to a chain of carbon atoms wherein all bonds between the carbon atoms in the alkyl group are single bonds. The term is inclusive

with respect to straight and branched chains (e.g., the term includes both n-propyl and isopropyl groups).

[0061] As used herein, the term “Cx-Cy alkyl” refers to an alkyl substituent with at least x carbon atoms and no more than y carbon atoms in the alkyl chain. For example, the term “C1-C3 alkyl” includes, without limitation, methyl, ethyl, n-propyl, and isopropyl substituents.

[0062] As used herein, the term “alkylene” refers to an alkyl chain that connects in at least two locations to other chemical groups. “Cx-Cy alkylene” refers to an alkylene substituent with at least x carbon atoms and no more than y carbon atoms in the alkylene chain. For example, the term “C1-C3 alkylene” includes, without limitation, methylene, ethylene, n-propylene, and iso-propylene.

[0063] As used herein, the term “alkenyl” refers to a chain of carbon atoms with at least one double bond between two carbon atoms in the chain. The term is inclusive with respect to straight and branched chains (e.g., the term includes both 1-propenyl and iso-propenyl groups).

[0064] As used herein, the term “Cx-Cy alkenyl” refers to an alkenyl substituent with at least x carbon atoms and no more than y carbon atoms in the alkenyl chain. For example, the term “C2-C4 alkenyl” includes, without limitation, vinyl and 1-propenyl.

[0065] As used herein, the term “alkenylene” refers to an alkenyl chain that connects in at least two locations to other chemical groups. “Cx-Cy alkenylene” refers to an alkenylene substituent with at least x carbon atoms and no more than y carbon atoms.

[0066] As used herein, the term “alkynyl” refers to a chain of carbon atoms with at least one triple bond between two carbon atoms in the chain. The term is inclusive with respect to straight and branched chains (e.g., the term includes both 1-propynyl and iso-propynyl groups).

[0067] As used herein, the term “Cx-Cy alkynyl” refers to an alkynyl substituent with at least x carbon atoms and no more than y carbon atoms in the alkynyl chain.

[0068] As used herein, the term “cycloalkyl” refers to a cyclic group of carbon atoms wherein all the bonds between the carbon atoms are single bonds. The term “Cx-Cy cycloalkyl” refers to a cycloalkyl substituent with at least x carbon atoms and no more than y carbon atoms. For example, the term “C6-C10 cycloalkyl” includes, without limitation, cyclohexyl and cyclo-octyl. The term “cycloalkylene” has the same meaning as cycloalkyl, except that the cycloalkylene substituent connects to at least two other chemical groups.

[0069] As used herein, the term “cycloalkenyl” refers to a cyclic group of carbon atoms where at least one bond between two carbon atoms in the cycloalkenyl group is a double bond. The term “Cx-Cy cycloalkenyl” refers to a cycloalkenyl substituent with at least x carbon atoms and no more than y carbon atoms.

[0070] As used herein, the term “aryl” refers to a cyclic group of carbon atoms with at least one double bond. The term “Cx-Cy aryl” refers to an arylene substituent with at least x carbon atoms and no more than y carbon atoms. For example, the term “C6-C10 aryl” includes, without limitation, phenyl and naphthyl. The term “arylene” has the same meaning as aryl, except that the arylene substituent connects to at least two other chemical groups.

[0071] As used herein, the term “heterocycloalkyl” refers to a cyclic group of atoms wherein all the bonds between the atoms in the ring are single bonds. The term “Cx-Cy heterocycloalkyl” refers to a heterocycloalkyl substituent with at least x atoms and no more than y atoms. For example, the term “C5-C6 heterocycloalkyl” includes, without limitation, pyrrolidinyl and 1,4-dioxanyl. The term “heterocycloalkylene” has the same meaning as heterocycloalkyl, except that the heterocycloalkylene substituent connects to at least two other chemical groups.

[0072] As used herein, the term “heteroaryl” refers to a cyclic group of atoms with at least one double bond. The term “x- to y-membered heteroaryl” refers to a cyclic group of atoms with at least x atoms and no more than y atoms. For example, 5- or 6-membered heteroaryl includes, without limitation, pyridinyl and furanyl.

[0073] As used herein, the term “carbocycle” refers to a substituent that can be a cycloalkyl or an aryl group. Likewise, the term “heterocycle” refers to a substituent that can be a heterocycloalkyl or a heteroaryl group.

[0074] Possible atoms that make up the ring in heterocycloalkyl and heteroaryl groups, as well as derivatives thereof, include, without limitation, carbon, nitrogen, oxygen, and sulfur.

[0075] As used herein, the term “optionally substituted” means the indicated substituent may be substituted or unsubstituted. The term substituted refers to another chemical moiety that decorates the indicated substituent by replacement of one H atom. For example, ethanol is an example of ethane substituted with OH. In some embodiments, a group that is optionally substituted is optionally substituted by chloro, fluoro, bromo, C1-C6 alkyl, C2-C6 alkenyl, C2-C6 alkynyl, C3-C7 cycloalkyl, C6-C10 aryl, or 5- or 6-membered heteroaryl.

[0076] The terms “individual,” “subject,” and “patient” are used interchangeably herein to describe a mammal, including humans. In some embodiments, the individual is in need

of treatment, for example, the individual may have been diagnosed with, or is suspected of having, a cancer.

[0077] It is understood that embodiments of the invention described herein include “consisting” and/or “consisting essentially of” embodiments.

[0078] Reference to “about” a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to “about X” includes description of “X”. In some embodiments, the term “about” a value or parameter means a range within 20%, in either direction, of the value or parameter recited.

[0079] As used herein, reference to “not” a value or parameter generally means and describes “other than” a value or parameter.

[0080] As used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise.

III. METHODS OF PREPARATION

[0081] Provided herein are methods of preparation of the modified RNA molecules disclosed herein.

A. *in vitro* Transcription

[0082] The modified RNA described herein can be synthesized through *in vitro* transcription of an appropriate DNA template. The promoter for controlling transcription can be any promoter for any RNA polymerase. A DNA template for *in vitro* transcription can be obtained, for example, by cloning of a nucleic acid, in particular cDNA, and introducing it into an appropriate vector for *in vitro* transcription. In some embodiments, the RNA may have modified nucleosides, including, for example, pseudouridine, and/or 1-methylpseudouridine.

[0083] In some embodiments, the appropriate DNA template for *in vitro* transcription comprises linearized plasmid DNA, PCR products, or synthetic DNA oligonucleotides. In some embodiments, the purity of the template DNA affects transcription yield and the integrity of RNA synthesized. In some embodiments, the template DNA has a purity of greater than about 90%, greater than about 95%, or greater than about 99%. Plasmid purification methods are commonly known in the art and include miniprep, midiprep, and maxiprep plasmid isolation. In some embodiments, the plasmid DNA is mostly in a supercoiled form, and is free from contaminating RNase, protein, RNA and salts.

[0084] In some embodiments, the DNA template for *in vitro* transcription comprises plasmid DNA that is completely linearized. In some embodiments, plasmid linearization is achieved through the action of a restriction enzyme downstream of the DNA template to be transcribed. In some embodiments the restriction enzyme generates blunt ends or 5'-overhangs. In some embodiments, following plasmid DNA linearization, the template DNA is further purified by phenol/chloroform extraction.

[0085] In some embodiments, the DNA template comprises a double-stranded T7 promoter region upstream of the sequence to be transcribed. In some embodiments, the promoter comprises an SP6, T7, or T3 promoter region. In some embodiments, the promoter is a phage polymerase promoter.

[0086] In some embodiments, described herein is a method of *in vitro* transcribing a DNA molecule to a modified RNA molecule, comprising combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, and wherein the 5' cap has the sequence of N1pppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine.

[0087] In some embodiments, described herein is a method of *in vitro* transcribing a DNA molecule to a modified RNA molecule, comprising combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, and wherein the 5' cap has the sequence of N1vpppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine.

[0088] In some embodiments, the *in vitro* transcription mix comprises a reaction buffer, modified and/or unmodified nucleotides (for example, modified and/or unmodified adenosines, guanosines, cytidines or uridines), a 5' cap, template DNA, a DNA-dependent RNA polymerase, and nuclease-free water. 1X Reaction buffer can include, for example, [40 mM Tris-HCl (pH 7.9-8.0), 10 mM DTT, 2 mM spermidine, 0.002% Triton X-100, 27 mM Magnesium Acetate]. In some embodiments, the reaction buffer comprises Tris-HCl (pH 7.9-8.0) at a concentration of between about 10mM and about 80 mM. In some embodiments, the reaction buffer comprises Tris-HCl (pH 7.9-8.0) at a concentration of about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, or about 80 mM. In some embodiments, the reaction buffer comprises Tris-

HCl (pH 7.9-8.0) at a concentration of about 40 mM. In some embodiments, the reaction buffer comprises DTT at a concentration of between about 1 mM and about 30 mM. In some embodiments, the reaction buffer comprises DTT at a concentration of about 1 mM, about 5 mM, about 10 mM, about 20 mM, or about 30 mM. In some embodiments, the reaction buffer comprises DTT at a concentration of about 10 mM. In some embodiments, the reaction buffer comprises spermidine at a concentration of between about 0.5 mM and about 10 mM. In some embodiments, the reaction buffer comprises spermidine at a concentration of about 0.5 mM, about 1 mM, about 2 mM, about 3 mM, about 5 mM, or about 10 mM. In some embodiments, the reaction buffer comprises spermidine at a concentration of about 2 mM. In some embodiments, the reaction buffer comprises Triton X-100 at a concentration of between about 0.0005% and about 0.05%. In some embodiments, the reaction buffer comprises Triton X-100 at a concentration of about 0.0005%, about 0.001%, about 0.002%, about 0.003%, about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.04% or about 0.05%. In some embodiments, the reaction buffer comprises Triton X-100 at a concentration of between about 0.0005% and about 0.005%. In some embodiments, the reaction buffer comprises Triton X-100 at a concentration of about 0.0005%, about 0.001%, about 0.002%, about 0.003%, about 0.004%, or about 0.005%. In some embodiments, the reaction buffer comprises Triton X-100 at a concentration of about 0.002%. In some embodiments, the reaction buffer comprises Magnesium Acetate at a concentration of between about 5 mM and about 100 mM. In some embodiments, the reaction buffer comprises Magnesium Acetate at a concentration of about 5 mM, about 10 mM, about 20 mM, about 27 mM, about 30 mM, about 40 mM, about 50 mM, about 75 mM, or about 100 mM. In some embodiments, the reaction buffer comprises Magnesium Acetate at a concentration of about 27 mM. Magnesium Chloride (MgCl_2) can be used instead of Magnesium Acetate. A similar effect can be achieved by adjusting the concentration of Magnesium Chloride.

[0089] In some embodiments, the final concentration of the modified and/or unmodified nucleotides in the *in vitro* transcription mix is between about 1 mM and about 100 mM. In some embodiments, the final concentration of the modified and/or unmodified nucleotides in the *in vitro* transcription mix is about 1 mM, about 5 mM, about 10 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM or about 100 mM. In some embodiments, the final concentration of the modified and/or unmodified nucleotides in the *in vitro* transcription mix is between about 1 mM and about 50 mM. In some embodiments, the final concentration of the modified and/or unmodified

nucleotides in the *in vitro* transcription mix is about 1 mM, about 5 mM, about 10 mM, about 12.5 mM, about 30 mM, about 40 mM, or about 50 mM. In some embodiments, the final concentration of the modified and/or unmodified nucleotides in the *in vitro* transcription mix is each about 10 mM. In some embodiments, the final concentration of the modified and/or unmodified nucleotides in the *in vitro* transcription mix is each about 5 mM. In some embodiments, the final concentration of the modified and/or unmodified nucleotides in the *in vitro* transcription mix is each about 12.5 mM. In some embodiments, the modified and/or unmodified nucleotides are added to the *in vitro* transcription mix at an equal ratio. In some embodiments, the 5' cap is added to the *in vitro* transcription mix at a concentration of between about 1 mM and about 50 mM. In some embodiments, the 5' cap is added to the *in vitro* transcription mix at a concentration of about 1 mM, about 2 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 12.5 mM, about 20 mM, about 30 mM, about 40 mM, or about 50 mM. In some embodiments, the 5' cap is added to the *in vitro* transcription mix at a concentration of about 8 mM. In some embodiments, the 5' cap is added to the *in vitro* transcription mix at a concentration of about 4 mM. In some embodiments, the 5' cap is added to the *in vitro* transcription mix at a concentration of about 12.5 mM. In some embodiments, the *in vitro* transcription mix is assembled at room temperature.

[0090] In some embodiments, the enzymatic capping step can occur co-transcriptionally. In some embodiments, the capping step occurs during the *in vitro* transcription of the modified RNA molecule. In some embodiments, the RNA cap is incorporated at the 5' end by RNA polymerases with relaxed substrate specificity thereby directly yielding the respective 5'-capped RNA. In some embodiments, the 5' cap included in this enzymatic capping step has the sequence of N1pppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine. In some embodiments, the 5' cap included in this enzymatic capping step has the sequence of N1vpppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine. In some embodiments, the 5' cap included in this enzymatic capping step has the sequence of GpppApU, wherein A is modified or unmodified. In some embodiments, the 5' cap included in this enzymatic capping step has the sequence of GvpppApU, wherein A is modified or unmodified. In some embodiments, the 5' cap included in this enzymatic capping step has the sequence of m⁷GpppApU, wherein A is modified or unmodified. In some embodiments,

the 5' cap included in this enzymatic capping step has the sequence of m⁷GvpppApU, wherein A is modified or unmodified. In some embodiments, the 5' cap included in this enzymatic capping step has the sequence of m⁷GpppApU, wherein the A is 2'-O-methylated. In some embodiments, the 5' cap included in this enzymatic capping step has the sequence of m⁷GpppA_mpU. In some embodiments, the 5' cap included in this enzymatic capping step has the sequence of m⁷GvpppApU, wherein the A is 2'-O-methylated. In some embodiments, the 5' cap included in this enzymatic capping step has the sequence of m⁷GvpppA_mpU.

[0091] In some embodiments, the *in vitro* transcription of modified RNAs comprises a capping step to produce the modified RNA comprising a 5' cap has the sequence of N1pppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine. In some embodiments the capping step is enzymatic or synthetic. In some embodiments, the 5' cap has the sequence of GpppApU, wherein A is modified or unmodified. In some embodiments, the 5' cap has the sequence of m⁷GpppApU. In some embodiments, the A is modified, preferably 2'-O-methylated. In some embodiments, the 5' cap has the sequence of m⁷GpppA_mpU.

[0092] In some embodiments, the *in vitro* transcription of modified RNAs comprises a capping step to produce the modified RNA comprising a 5' cap has the sequence of N1vpppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine. In some embodiments the capping step is enzymatic or synthetic. In some embodiments, the 5' cap has the sequence of GvpppApU, wherein A is modified or unmodified. In some embodiments, the 5' cap has the sequence of m⁷GvpppApU. In some embodiments, the A is modified, preferably 2'-O-methylated. In some embodiments, the 5' cap has the sequence of m⁷GvpppA_mpU.

[0093] In some embodiments, the *in vitro* transcription mix comprises modified adenosines, guanosines, cytidines or uridines. In some embodiments, the *in vitro* transcription mix comprises unmodified adenosines, guanosines, cytidines or uridines. In some embodiments, the *in vitro* transcription mix comprises modified adenosines, guanosines, cytidines and uridines. In some embodiments, the *in vitro* transcription mix comprises unmodified adenosines, guanosines, cytidines and uridines. In some embodiments, the *in vitro* transcription mix comprises unmodified adenosines, guanosines, and cytidines. In some embodiments, the *in vitro* transcription mix comprises modified

uridines. In some embodiments, the modified uridine comprises Ψ or m1 Ψ . In some embodiments, the modified uridine is m1 Ψ .

[0094] In some embodiments, between about 1% and about 99% of the uridine bases in the modified RNA molecule are modified. In some embodiments, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% of the uridines in the RNA molecule are modified. In some embodiments, at least one uridine in the modified RNA molecule is a modified uridine except for the first 5' uridine. In some embodiments, at least about 50% of the uridines other than the first 5' uridine in the molecule are modified. In some embodiments, at least about 90% of the uridines other than the first 5' uridine in the molecule are modified. In some embodiments, at least about 95% of the uridines other than the first 5' uridine in the molecule are modified. In some embodiments, at least about 99% of the uridines other than the first 5' uridine in the molecule are modified.

[0095] In some embodiments, all of the uridines in the molecule are modified except for the first 5' uridine.

[0096] In some embodiments, all of the uridines in the molecule are m1 Ψ except for the first 5' uridine.

[0097] In some embodiments, the method of *in vitro* transcribing a DNA molecule to a modified RNA molecule comprises combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, wherein the 5' cap has the sequence of N1pppN2pU or N1vpppN2pU, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine, wherein the *in vitro* transcription mix comprises modified uridines (preferably m1 Ψ), and wherein at least one uridine in the modified RNA molecule is a modified uridine except for the first 5' uridine.

[0098] In some embodiments, the method of *in vitro* transcribing a DNA molecule to a modified RNA molecule comprises combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, wherein the 5' cap has the sequence of N1pppN2pU or N1vpppN2pU, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine, wherein the *in vitro*

transcription mix comprises modified uridines (preferably m1Ψ), and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0099] In a preferred embodiment, the method of *in vitro* transcribing a DNA molecule to a modified RNA molecule comprises combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, wherein the 5' cap has the sequence of GpppApU or GvpppApU, wherein U is an unmodified uridine, wherein the *in vitro* transcription mix comprises modified uridines (preferably m1Ψ), and wherein at least one uridine in the modified RNA molecule is a modified uridine except for the first 5' uridine.

[0100] In a preferred embodiment, the method of *in vitro* transcribing a DNA molecule to a modified RNA molecule comprises combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, wherein the 5' cap has the sequence of GpppApU or GvpppApU, wherein U is an unmodified uridine, wherein the *in vitro* transcription mix comprises modified uridines (preferably m1Ψ), and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0101] In a preferred embodiment, the method of *in vitro* transcribing a DNA molecule to a modified RNA molecule comprises combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, wherein the 5' cap has the sequence of m⁷GpppApU or m⁷GvpppApU, wherein U is an unmodified uridine, wherein the *in vitro* transcription mix comprises modified uridines (preferably m1Ψ), and wherein at least one uridine in the modified RNA molecule is a modified uridine except for the first 5' uridine.

[0102] In a preferred embodiment, the method of *in vitro* transcribing a DNA molecule to a modified RNA molecule comprises combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, wherein the 5' cap has the sequence of m⁷GpppApU or m⁷GvpppApU, wherein U is an unmodified uridine, wherein the *in vitro* transcription mix comprises modified

uridines (preferably m¹Ψ), and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0103] In a more preferred embodiment, the method of *in vitro* transcribing a DNA molecule to a modified RNA molecule comprises combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, wherein the 5' cap has the sequence of m⁷GpppA_mpU or m⁷GvpppA_mpU, wherein U is an unmodified uridine, wherein the *in vitro* transcription mix comprises modified uridines (preferably m¹Ψ), and wherein at least one uridine in the modified RNA molecule is a modified uridine except for the first 5' uridine.

[0104] In a more preferred embodiment, the method of *in vitro* transcribing a DNA molecule to a modified RNA molecule comprises combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, wherein the 5' cap has the sequence of m⁷GpppA_mpU or m⁷GvpppA_mpU, wherein U is an unmodified uridine, wherein the *in vitro* transcription mix comprises modified uridines (preferably m¹Ψ), and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0105] In some embodiments, the *in vitro* transcription mix comprises a DNA-dependent RNA polymerase. In some embodiments, the DNA-dependent RNA polymerase is a T7 RNA polymerase, an SP3 RNA polymerase, an SP6 RNA polymerase, a VSW-3 RNA polymerase, or variants thereof. In some embodiments, the DNA-dependent RNA polymerase is a T7 RNA polymerase or the variant thereof. In some embodiments, the DNA-dependent RNA polymerase is a T7 RNA polymerase.

[0106] In some embodiments, the *in vitro* transcription reaction comprises an incubation step. In some embodiments, the incubation time is between about 5 minutes and about 360 minutes. In some embodiments, the incubation time is about 20 minutes, about 40 minutes, about 60 minutes, about 80 minutes, about 100 minutes, about 120 minutes, about 140 minutes, about 150 min, about 160 minutes, about 180 minutes, about 200 minutes, about 220 minutes, about 240 minutes, about 260 minutes, about 280 minutes, about 300 minutes, about 320 minutes, about 340 minutes, or about 360 minutes. In some embodiments, the incubation time is greater than about 360 minutes. In some embodiments, the incubation time is between about 5 minutes and about 140 minutes. In some embodiments, the incubation time is about 20minutes, about 40 minutes, about 60 minutes,

about 80 minutes, about 100 minutes, about 120 minutes, or about 140 minutes. In some embodiments, the incubation time is greater than about 140 minutes. In some embodiments, the incubation is performed at a temperature of between about 30°C and about 42°C. In some embodiments, the incubation is performed at about 30°C, about 31°C, about 32°C, about 33°C, about 34°C, about 35°C, about 36°C, about 37°C, about 38°C, about 39°C, about 40°C, about 41°C, or about 42°C. In some embodiments, the incubation is performed at a temperature of between about 35°C and about 40°C. In some embodiments, the incubation is performed at about 35°C, about 36°C, about 37°C, about 38°C, about 39°C, or about 40°C. In some embodiments, the incubation step comprises a 2 hour incubation at 37°C. In some embodiments, the incubation is performed in a water bath, heating block, dry air incubator, or PCR instrument.

[0107] In some embodiments, the products of the *in vitro* transcription reaction are evaluated to detect RNA purity and capping efficiency. In some embodiments, RNA concentration is quantified by UV light absorbance. In some embodiments, RNA concentration is determined by measuring the ultraviolet light absorbance at 260 nm wavelength. In some embodiments, free nucleotides from the transcription reaction are removed before quantifying RNA concentration by ultraviolet light absorbance. In some embodiments, the transcription products of the *in vitro* transcription reaction are analyzed by gel electrophoresis. In some embodiments, transcript length, integrity, and/or quantity is analyzed by running an aliquot of the transcription reaction on an appropriate denaturing agarose gel or polyacrylamide gel. In some embodiments, transcripts larger than 0.3 kb can be run on agarose gels. In some embodiments, transcripts smaller than about 0.3 kb are run on denaturing polyacrylamide gels. In some embodiments, the percent polyacrylamide in the polyacrylamide gel is between about 5% and about 15%.

[0108] Also provided herein are methods to isolate the modified RNA molecule. In some embodiments, the modified RNA molecule is isolated by chemical-, column-, and/or gel-based approaches. Alternatively, in some embodiments, the modified RNA molecule is isolated by bead-based methods. In some embodiments, the bead-based methods isolate the modified RNA molecule through binding of the poly (A) tail. In some embodiments, the methods to isolate the modified RNA molecules described herein are used to clean up and separate the RNA from components such as DNA templates, unincorporated nucleotides, or RNA modifying enzymes.

IV. MODIFIED RNA MOLECULE

[0109] Also provided herein are RNA molecules generated by the methods described herein. Thus, in certain aspects, provided herein are modified RNA molecules comprising a 5' cap, wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine in the molecule. In some embodiments, the modified RNA molecule is not self-replicable.

[0110] In some embodiments, the modified RNA molecule comprises the nitrogenous bases guanine, uracil, adenine, and cytosine. In some embodiments, the modified RNA molecule comprises modified nitrogenous bases. In some embodiments, the modified RNA molecule comprises at least one modified G, C, or A. In some embodiments, the modified RNA molecule comprises at least one modified U. In some embodiments the modified U comprises Ψ (pseudouridine). In some embodiments the modified U comprises m 1Ψ (1-methyl-pseudouridine).

[0111] In some embodiments, the 5' end of the modified RNA molecule has the sequence of N1pppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine. In some embodiments, between about 1% and about 99% of the uridine bases in the modified RNA molecule are modified. In some embodiments, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% of the uridines in the RNA molecule are modified. In some embodiments, at least about 50% of the uridines other than the first 5' uridine in the molecule are modified. In some embodiments, at least about 90% of the uridines other than the first 5' uridine in the molecule are modified. In some embodiments, at least about 95% of the uridines other than the first 5' uridine in the molecule are modified. In some embodiments, at least about 99% of the uridines other than the first 5' uridine in the molecule are modified. In some embodiments, all of the uridines other than the first 5' uridine in the molecule are modified.

[0112] In some embodiments, the 5' end of the modified RNA molecule has the sequence of N1pppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine. In some embodiments, between about 1% and about 99% of the uridine bases in the modified RNA molecule are Ψ . In some embodiments, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% of the uridines in the RNA molecule are Ψ . In some embodiments, at

least about 50% of the uridines other than the first 5' uridine in the molecule are Ψ . In some embodiments, at least about 90% of the uridines other than the first 5' uridine in the molecule are Ψ . In some embodiments, at least about 95% of the uridines other than the first 5' uridine in the molecule are Ψ . In some embodiments, at least about 99% of the uridines other than the first 5' uridine in the molecule are Ψ . In some embodiments, all of the uridines other than the first 5' uridine in the molecule are Ψ .

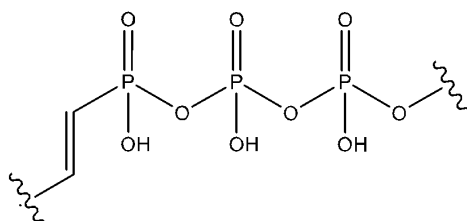
[0113] In some embodiments, the 5' end of the modified RNA molecule has the sequence of N1pppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine. In some embodiments, between about 1% and about 99% of the uridine bases in the modified RNA molecule are m1 Ψ . In some embodiments, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% of the uridines in the RNA molecule are m1 Ψ . In some embodiments, at least about 50% of the uridines other than the first 5' uridine in the molecule are m1 Ψ . In some embodiments, at least about 90% of the uridines other than the first 5' uridine in the molecule are m1 Ψ . In some embodiments, at least about 95% of the uridines other than the first 5' uridine in the molecule are m1 Ψ . In some embodiments, at least about 99% of the uridines other than the first 5' uridine in the molecule are m1 Ψ . In some embodiments, all of the uridines other than the first 5' uridine in the molecule are m1 Ψ .

[0114] In some embodiments, the 5' end of the modified RNA molecule has the sequence of N1vpppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine. In some embodiments, between about 1% and about 99% of the uridine bases in the modified RNA molecule are modified. In some embodiments, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% of the uridines in the RNA molecule are modified. In some embodiments, at least about 50% of the uridines other than the first 5' uridine in the molecule are modified. In some embodiments, at least about 90% of the uridines other than the first 5' uridine in the molecule are modified. In some embodiments, at least about 95% of the uridines other than the first 5' uridine in the molecule are modified. In some embodiments, at least about 99% of the uridines other than the first 5' uridine in the molecule are modified. In some embodiments, all of the uridines other than the first 5' uridine in the molecule are modified.

[0115] In some embodiments, the 5' end of the modified RNA molecule has the sequence of N1vpppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine. In some embodiments, between about 1% and about 99% of the uridine bases in the modified RNA molecule are Ψ . In some embodiments, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% of the uridines in the RNA molecule are Ψ . In some embodiments, at least about 50% of the uridines other than the first 5' uridine in the molecule are Ψ . In some embodiments, at least about 90% of the uridines other than the first 5' uridine in the molecule are Ψ . In some embodiments, at least about 95% of the uridines other than the first 5' uridine in the molecule are Ψ . In some embodiments, at least about 99% of the uridines other than the first 5' uridine in the molecule are Ψ . In some embodiments, all of the uridines other than the first 5' uridine in the molecule are Ψ .

[0116] In some embodiments, the 5' end of the modified RNA molecule has the sequence of N1vpppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine. In some embodiments, between about 1% and about 99% of the uridine bases in the modified RNA molecule are m1 Ψ . In some embodiments, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% of the uridines in the RNA molecule are m1 Ψ . In some embodiments, at least about 50% of the uridines other than the first 5' uridine in the molecule are m1 Ψ . In some embodiments, at least about 90% of the uridines other than the first 5' uridine in the molecule are m1 Ψ . In some embodiments, at least about 95% of the uridines other than the first 5' uridine in the molecule are m1 Ψ . In some embodiments, at least about 99% of the uridines other than the first 5' uridine in the molecule are m1 Ψ . In some embodiments, all of the uridines other than the first 5' uridine in the molecule are m1 Ψ .

[0117] The triphosphate linkage of the N1vpppN2pU is vinyl-modified compared to that of the N1pppN2pU. For example, N1vpppN2pU contains the vinyl-modified triphosphate linkage, comprising the following moiety.



[0118] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of N1pppN2pU, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine, and wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine.

[0119] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of N1pppN2pU, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine, and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0120] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of N1vpppN2pU, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine, and wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine.

[0121] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of N1vpppN2pU, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine, and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0122] In some embodiments the modified RNA molecule is involved in protein synthesis. In some embodiments the modified RNA molecule is an mRNA. In some embodiments, the modified RNA molecule comprises a protein coding open reading frame (ORF).

[0123] In some embodiments, the length of the modified RNA molecule is at least 20 nucleotides. In some embodiments, the length of the modified RNA molecule is between about 20 nucleotides and about 8000 nucleotides. In some embodiments, the length of the RNA molecule is at least about 20 nucleotides, about 50 nucleotides, about 100 nucleotides, about 200 nucleotides, about 300 nucleotides, about 400 nucleotides, about 500 nucleotides, about 750 nucleotides, about 1000 nucleotides, about 1500 nucleotides, about 2000

nucleotides, about 3000 nucleotides, about 4000 nucleotides, about 6000 nucleotides, about 7000 nucleotides, or about 8000 nucleotides.

[0124] In some embodiments, the modified RNA molecule comprises non-coding elements. In some embodiments the non-coding elements comprise a 5' 7-methyl-GTP cap, a 5' untranslated region (UTR), and a 3' UTR. In some embodiments, the mRNA comprises a 5' UTR. The 5' UTRs provided herein may be recognized by the ribosome, thereby allowing the ribosome to bind and initiate translation of the mRNA (e.g., translation of the coding sequence and/or nucleic acid encoding a signal peptide of the mRNA). In some embodiments, the 5' UTR is upstream from the coding sequence of the mRNA. In some embodiments, the 5' UTR is between about 5 and 1400 nucleotides long. In some embodiments, the 5' UTR is between about 5 to about 20, about 20 to about 40, about 40 to about 60, about 60 to about 80, about 80 to about 100, about 100 to about 150, about 150 to about 200, about 200 to about 250, about 250 to about 300, about 300 to about 400, or about 400 to about 500 nucleotides long. In some embodiments the 5' UTR is about 66 nucleotides long.

[0125] In some embodiments, the mRNA comprises a 5' UTR and a 3' UTR, such as any of the 5' UTRs and 3' UTRs provided herein. In some embodiments, the 5' UTR and the 3' UTR are derived from the same species. In some embodiments, the 5' UTR and the 3' UTR are not derived from the same species. In some embodiments, the 5' UTR is synthetic, and the 3' UTR is not synthetic. In some embodiments, the 5' UTR is not synthetic, and the 3' UTR is synthetic.

[0126] In some embodiments, the mRNA comprises a poly (A) sequence (e.g., a polyadenylation sequence). Poly (A) sequences consist of multiple adenosine monophosphates in succession. In some embodiments, the poly (A) sequence is crucial for translation of the mRNA. In some embodiments, the poly (A) sequence is downstream of the coding sequence of the mRNA. In some embodiments, the poly (A) sequence is downstream of a 3' UTR of the mRNA. In some embodiments, the poly(A) sequence has a length of about 50 nucleotides or longer, such as about 60 nucleotides, 70 nucleotides, 80 nucleotides, 90 nucleotides, 100 nucleotides, 150 nucleotides, or longer. In some embodiments, the poly(A) sequence has a length of about 150 nucleotides or shorter, such as about 100 nucleotides, 90 nucleotides, 80 nucleotides, 70 nucleotides, 50 nucleotides, or shorter. In some embodiments, the poly(A) sequence has a length of about 105 nucleotides.

[0127] In some embodiments, the modified RNA molecule is not self-replicable. In some embodiments, the modified RNA molecule degrades over time in cells. In some

embodiments degradation of the mRNA in cells occurs through mRNA decay, AU-rich elements (AREs) in the 3' UTR, destabilizing elements in protein-coding regions, nonsense-mediated mRNA decay (NMD), and/or microRNAs (miRNAs). In some embodiments, the modified RNA molecule has a measurable half-life in cells. In some embodiments, the half-life of the modified RNA molecule in cells is between about 0 hours and about 24 hours. In some embodiments, the half-life of the modified RNA molecule in cells is between about 0 hours to 1 hour, about 1 hour to 2 hours, about 2 hours to about 3 hours, about 3 hours to about 4 hours, about 5 hours to about 7 hours, about 7 hours to about 9 hours, about 9 hours to about 11 hours, about 11 hours to about 15 hours, about 15 hours to about 20 hours, about 20 hours to about 24 hours. In some embodiments, the half-life of the modified RNA molecule in cells is about 10 hours.

[0128] In some embodiments, the modified RNA molecule can be transfected into a cell to be translated intracellularly. Methods of transfection are known to those of skill in the art and include microinjection, chemical treatments, and electroporation. In some embodiments the cell to be transfected comprises any patient cell for which it is desired to express a protein of interest. In some embodiments, the cell to be transfected includes vascular endothelial cells, epidermal cells, bronchial endothelial cells, adipocytes, dermal fibroblasts, muscle cells, and hematopoietic cells (e.g., T cells, B cells, dendritic cells, macrophages, etc.), germ cells, or tissue culture cells.

A. 5' Cap

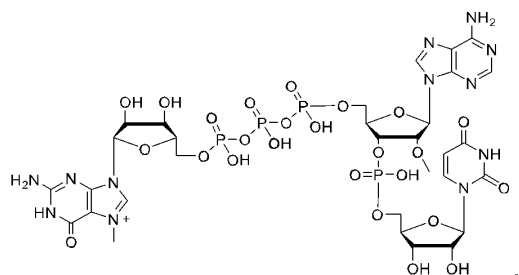
[0129] In some embodiments, the modified RNA molecule comprises a 5' cap.

[0130] In some embodiments, the 5' end of the modified RNA molecule has the sequence of N1pppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine.

[0131] In some embodiments, the 5' end of the modified RNA molecule has the sequence of GpppApU, wherein A is modified or unmodified. In some embodiments, the A is 2'-O-methylated. In some embodiments, the 5' end of the modified RNA molecule has the sequence of m⁷GpppApU. In some embodiments, the 5' end of the modified RNA molecule has the sequence of m⁷GpppApU, wherein the A is modified or unmodified. In some embodiments, the A is 2'-O-methylated. In some embodiments, the 5' end of the modified RNA molecule has the sequence of m⁷GpppA_mpU. In some embodiments, the 5' end of the modified RNA molecule comprises a nucleic acid sequence set forth in any one

of SEQ ID NOs: 2, 4, 6, or 8. In some embodiments, the 5' end of the modified RNA molecule comprises a nucleic acid sequence set forth in any one of SEQ ID NOs: 9, 12, 15, 18, 22, 25, 28 or 31. In some embodiments, the 5' end of the modified RNA molecule comprises a nucleic acid sequence set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 9, 12, 15, 18, 22, 25, 28 or 31.

[0132] In some embodiments, $m^7GpppA_{mp}U$ has the following formula,



[0133] In some embodiments, a modified RNA molecule comprising a 5' cap comprising the sequence of $m^7GpppA_{mp}U$ displays increased RNA stability compared with an unmodified RNA.

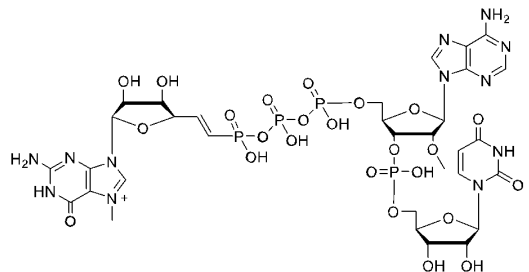
[0134] In some embodiments, the 5' end of the modified RNA molecule has the sequence of $N1vpppN2pU$, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine.

[0135] In some embodiments, the 5' end of the modified RNA molecule has the sequence of $GvpppApU$, wherein A is modified or unmodified. The triphosphate linkage of the $GvpppApU$ is vinyl-modified compared to that of the $GpppApU$. In some embodiments, the A is 2'-O-methylated. In some embodiments, the 5' end of the modified RNA molecule has the sequence of $m^7GvpppApU$. The triphosphate linkage of the $m^7GvpppApU$ is vinyl-modified compared to that of the $m^7GpppApU$. In some embodiments, the 5' end of the modified RNA molecule has the sequence of $m^7GvpppApU$, wherein the A is modified or unmodified. In some embodiments, the A is 2'-O-methylated. In some embodiments, the 5' end of the modified RNA molecule has the sequence of $m^7GvpppA_{mp}U$. The triphosphate linkage of the $m^7GvpppA_{mp}U$ is vinyl-modified compared to that of the $m^7GpppA_{mp}U$. In some embodiments, the 5' end of the modified RNA molecule comprises a nucleic acid sequence set forth in any one of SEQ ID NOs: 21 or 34.

[0136] In some embodiments, a modified RNA molecule comprising a 5' cap comprising the sequence of $m^7GvpppA_{mp}U$ displays increased RNA stability compared with an unmodified RNA. Additionally, the double bond structure makes the molecule

conformation more stable and makes it more difficult for nucleases to identify and hydrolyze.

[0137] In some embodiments, $m^7GvpppA_m pU$ has the following formula,



[0138] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of GpppApU, wherein U is an unmodified uridine, and wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine.

[0139] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of GpppApU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0140] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of GpppApU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are Ψ except for the first 5' uridine.

[0141] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of GpppApU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are $m^1\Psi$ except for the first 5' uridine.

[0142] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of $m^7GvpppApU$, wherein U is an unmodified uridine, and wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine.

[0143] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of $m^7GvpppApU$, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0144] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GpppApU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are Ψ except for the first 5' uridine.

[0145] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GpppApU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are m¹Ψ except for the first 5' uridine.

[0146] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GpppA_mpU, wherein U is an unmodified uridine, and wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine.

[0147] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GpppA_mpU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0148] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GpppA_mpU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are Ψ except for the first 5' uridine.

[0149] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GpppA_mpU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are m¹Ψ except for the first 5' uridine.

[0150] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of GvpppApU, wherein U is an unmodified uridine, and wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine.

[0151] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of GvpppApU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0152] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule

has the sequence of GvpppApU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are Ψ except for the first 5' uridine.

[0153] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of GvpppApU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are m¹ Ψ except for the first 5' uridine.

[0154] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GvpppApU, wherein U is an unmodified uridine, and wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine.

[0155] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GvpppApU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0156] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GvpppApU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are Ψ except for the first 5' uridine.

[0157] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GvpppApU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are m¹ Ψ except for the first 5' uridine.

[0158] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GvpppA_mpU, wherein U is an unmodified uridine, and wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine.

[0159] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GvpppA_mpU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are modified except for the first 5' uridine.

[0160] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GvpppA_mpU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are Ψ except for the first 5' uridine.

[0161] In some embodiments, the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GvpppA_mpU, wherein U is an unmodified uridine, and wherein all of the uridines in the molecule are m¹Ψ except for the first 5' uridine.

[0162] In some embodiments, a modified RNA molecule comprising a 5' cap that is significantly less immunostimulative than an unmodified RNA molecule. In some embodiments, the modified RNA molecule is at least 2-fold less immunostimulative than an unmodified RNA molecule, or a modified RNA molecule containing a different 5' cap, e.g., a 5' cap not containing an unmodified uridine. In some embodiments, the modified RNA molecule is between about 2-fold to 20-fold less immunostimulative than an unmodified RNA molecule, or a modified RNA molecule containing a different 5' cap, e.g., a 5' cap not containing an unmodified uridine. In some embodiments, the modified RNA molecule is about 1.2-fold, about 1.4-fold, about 1.5-fold, about 1.6-fold, about 1.7-fold, about 1.8-fold, about 1.9-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 10-fold, or about 20-fold less immunostimulative than an unmodified RNA molecule, or a modified RNA molecule containing a different 5' cap, e.g., a 5' cap not containing an unmodified uridine. In some embodiments, the modified RNA molecule is about 1.4-fold less immunostimulative than an unmodified RNA molecule, or a modified RNA molecule containing a different 5' cap, e.g., a 5' cap not containing an unmodified uridine. In some embodiments, the modified RNA molecule is about 1.9-fold less immunostimulative than an unmodified RNA molecule, or a modified RNA molecule containing a different 5' cap, e.g., a 5' cap not containing an unmodified uridine.

[0163] In some embodiments, "significantly less immunostimulative" refers to a detectable decrease in immunostimulation. In another embodiment, the term refers to a fold decrease in immunostimulation. In some embodiments, "significantly less immunostimulative" refers to a decrease such that an effective amount of the modified RNA molecule can be administered to cells without triggering a detectable immune response. In some embodiments, the term refers to a decrease such that the modified RNA molecule can be repeatedly administered without eliciting an immune response sufficient to detectably reduce expression of the recombinant protein. In another embodiment, the decrease is such that the modified RNA molecule can be repeatedly administered without eliciting an immune response sufficient to eliminate detectable expression of the recombinant protein. Methods of determining immunostimulation are well known in the art, and include, e.g. measuring secretion of cytokines (e.g. IL-12, IFN-α, TNF-α, RANTES, MIP-1α or β, IL-6,

IFN- β , or IL-8), measuring expression of DC activation markers (e.g. CD83, HLA-DR, CD80 and CD86), or measuring the ability to act as an adjuvant for an adaptive immune response. In some embodiments, the immunostimulation of the modified RNA molecule is measured by a RIG-I activation assay.

B. Modifications

[0164] Other than the first uridine (e.g., the uridine introduced by the 5' cap), the RNA molecule can comprise one or more modifications. In some embodiments, the modification comprises modified nucleosides. In some embodiments, the modified nucleoside is uridine (U). In some embodiments, the modified nucleoside is adenine (A). In some embodiments, the modified nucleoside is cytidine (C). In some embodiments, the modified nucleoside is guanine (G). In some embodiments, the nucleoside is a non-canonical nucleoside such as, for example, inosine.

[0165] In some embodiments, the modified RNA molecule may comprise modified base forms. In some embodiments, purines and pyrimidines other than those normally found in nature may be utilized. In some embodiments, the modified base is selected from the group consisting of m⁵C (5-methylcytidine), m⁵U (5-methyluridine), m⁶A (N6-methyladenosine), S²U (2-thiouridine), Ψ (pseudouridine), Um (2'-O-methyluridine), Hi¹A (1-methyladenosine), Hi²A (2-methyladenosine), Am (2'-O-methyladenosine), ms²m⁶A (2-methylthio-N6-methyladenosine), i⁶A (N6-isopentenyladenosine), ms²i⁶A (2-methylthio-N6isopentenyladenosine), io⁶A (N6-(cis-hydroxyisopentenyl)adenosine), ms²io⁶A (2-methylthio-N6-(cis-hydroxyisopentenyl) adenosine), g⁶A (N6-glycinylnorvalyladenosine), t⁶A (N6-threonylnorvalyladenosine), ms²t⁶A (2-methylthio-N6-threonyl carbamoyl-adenosine), m⁶t⁶A (N6-methyl-N6-threonylnorvalyladenosine), Im⁶A (N6-hydroxy norvalylcarbamoyl-adenosine), ms²hn⁶A (2-methylthio-N6-hydroxynorvalyl carbamoyl-adenosine), Ar(p) (2'-O-ribosyladenosine (phosphate)), I (inosine), Hi¹I (1-methylinosine), Hi¹Im (1,2'-O-dimethylinosine), m³C (3-methylcytidine), Cm (2'-O-methylcytidine), S²C (2-thiocytidine), ac⁴C (N4-acetylcytidine), f⁵C (5-formylcytidine), m⁵Cm (5,2'-O-dimethylcytidine), ac⁴Cm (N4-acetyl-2'-O-methylcytidine), k²C (lysidine), Hi¹G (1-methylguanosine), m²G (N2-methylguanosine), m⁷G (7-methylguanosine), Gm (2'-O-methylguanosine), m²²G (N2,N2-dimethylguanosine), m²Gm (N2,2'-O-dimethylguanosine), m²²Gm (N2,N2,2'-O-trimethylguanosine), Gr(p) (2'-O-ribosylguanosine (phosphate)), yW (wybutosine), o²yW (peroxywybutosine), OHyW (hydroxy wybutosine), OHyW* (undermodified

hydroxywybutosine), imG (wyosine), mimG (methyl wyosine), Q (queuosine), oQ (epoxyqueuosine), galQ (galactosyl-queuosine), manQ (mannosyl- queuosine), preQ0 (7-cyano-7-deazaguanosine), preQi (7-aminomethyl-7-deazaguanosine), G⁺ (archaeosine), D (dihydrouridine), m5Um (5,2'-O-dimethyluridine), S⁴U (4-thiouridine), m⁵s²U (5- methyl-2-thiouridine), S²Um (2-thio-2'-O-methyluridine), acp³U (3-(3-amino-3-carboxypropyl)uridine), ho⁵U (5-hydroxyuridine), mo5U (5-methoxyuridine), cmo⁵U (uridine 5-oxyacetic acid), mcmo5U (uridine 5-oxyacetic acid methyl ester), chm⁵U (5-(carboxyhydroxymethyl)uridine)), mchm⁵U (5- (carboxyhydroxymethyl)uridine methyl ester), mcm⁵U (5-methoxycarbonylmethyluridine), mcm5Um (5-methoxycarbonylmethyl-2'-O-metliyluridine), mcm⁵s²U (5-methoxycarbonylmethyl-2-thiouridine), nmVU (5-aminomethyl-2-thiouridine), mnm5U (5-methylaminomethyluridine), mnm⁵s²U (5-methylaminomethyl-2-thiouridine), mnm⁵se²U (5-methylaminomethyl-2-selenouridine), ncm⁵U (5- carbamoylmethyluridine), ncm⁵Um (5-carbamoylmethyl-2'-O-methyluridine), cmnm⁵U (5- carboxy methylaminomethyluridine), cmnm⁵Um (5-carboxymethylaminomethyl-2'-0-methyluridine), cmnm⁵s²U (5-carboxymethylaminomethyl-2-thiouridine), m⁶2A (N6,N6-dimethyladenosine), Im (2'-O-methylinosine), m⁴C (N4-methylcytidine), m⁴Cm (N4,2'-O-dimethylcytidine), hm⁵C (5-hydroxymethylcytidine), m³U (3-methyluridine), Cm⁵U (5-carboxymethyluridine), m⁶Am (N5,2'-O- dimethyladenosine), m⁶2Am (N6,N6,O-2t-trimethyladenosine), m^{2,7}G (N²,7-dimethylguanosine), m^{2,2,7}G (N2,N2,7-trimethylguanosine), m³Um (3,2'-O-dimethyluridine), m⁵D (5-methyldihydrouridine), f⁵Cm (5-formyl-2'-O-methylcytidine), Hi¹Gm (1,2'-O-dimethylguanosine), m¹Am (1,2'-O-dimethyladenosine), tm⁵U (5-taurinomethyluridine); tm⁵s²U (5-taurinomethyl-2-thiouridine)), imG-14 (4-demethylwyosine), imG² (isowyosine), and ac⁶A (N6-acetyladenosine).

[0166] In some embodiments, the modified base comprises pseudouridine (5-ribosyluracil) (Ψ). In some embodiments, pseudouridine alters base-pairing interactions thereby affecting RNA secondary structures and mRNA coding. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine (m1Ψ). In some embodiments, Ψ and m1Ψ, when present in an *in vitro* transcribed mRNA within a host cell, reduce immunostimulation of the modified RNA.

[0167] In some embodiments, between about 0.5% and 99% of the uridines in the modified RNA molecule are modified. In some embodiments, between about 0.5% to about 10%, about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about

80%, about 80% to about 90%, or about 90% to about 99% of the uridines in the modified RNA molecule are modified. In some embodiment, at least one uridine in the molecule is a modified uridine except for the first 5' uridine. In some embodiments, at least about 50% of the uridines other than the first 5' uridine in the molecule are modified. In some embodiments, at least about 50% of the uridines other than the first 5' uridine in the molecule are Ψ . In some embodiments, at least about 50% of the uridines other than the first 5' uridine in the molecule are m1 Ψ .

[0168] In some embodiments, all of the uridines in the molecule are modified except for the first 5' uridine. In some embodiments, all of the uridines in the molecule are Ψ modified except for the first 5' uridine. In some embodiments, all of the uridines in the molecule are m1 Ψ except for the first 5' uridine.

[0169] In some embodiments, the modified RNA molecule comprises one or more modifications to the phosphate backbone and/or modifications to the sugars. In some embodiments, the modification is selected from the group consisting of MOE, 2'-OMe, LNA, GalNAc, 5' methylcytosine, a phosphorothioate bond, an alkylphosphonate bond, a phosphoroamidate bond, a boranophosphate bond, and/or a morpholino ring.

C. Coding Sequence

[0170] In some embodiments, the modified RNA molecule comprises an open reading frame comprising a coding sequence. In some embodiments, the open reading frame encodes a recombinant protein. In some embodiments, the open reading frame encodes one or more recombinant proteins. In some embodiments, the open reading frame encodes a protein of interest. In some embodiments, the protein of interest is the only protein encoded in the coding sequence.

[0171] In some embodiments, the open reading frame encodes any known amino acid. In some embodiments, the open reading frame encodes amino acids selected from the group consisting of alanine, arginine, asparagine, aspartate, cysteine, glutamine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, selenocysteine, and pyrrolysine.

[0172] In some embodiments, the open reading frame encodes a recombinant protein. In some embodiments, the recombinant protein is selected from the group consisting of enzymes, structural proteins, transport proteins, receptors, hormones, antibodies, transcription factors, growth factors, cytokines, motor proteins, structural proteins, and chaperones. In some embodiments, the enzyme is selected from the group consisting of

oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, polymerases, kinases, phosphatases, proteases, lipases and amylases.

[0173] In some embodiments, the open reading frame encodes a therapeutic payload, which comprises a compound capable of eliciting immunity against one or more target conditions or diseases. In some embodiments, the target condition is related to or caused by infection by a pathogen, such as a coronavirus (e.g., 2019-nCoV), influenza, measles, human papillomavirus (HPV), rabies, meningitis, whooping cough, tetanus, plague, hepatitis, and tuberculosis. In some embodiments, the open reading frame encodes a pathogenic protein characteristic for the pathogen, or an antigenic fragment or epitope thereof. In some embodiments, the target condition is related to or caused by neoplastic growth of cells, such as a cancer. In some embodiments, the open reading frame encodes a tumor-associated antigen (TAA) characteristic for the cancer, or an antigenic fragment or epitope thereof.

[0174] In some embodiments, the open reading frame encodes a reporter protein. In some embodiments, the reporter protein is a fluorescent protein. In some embodiments, the reporter protein is eGFP. In some embodiments, the open reading frame encoding a reporter protein comprises a sequence set forth in SEQ ID NOs: 1-2. In some embodiments, the open reading frame encoding a reporter protein comprises a sequence set forth in SEQ ID NOs: 18-21 or 31-34.

[0175] In some embodiments, the open reading frame encodes a viral protein. In some embodiments, the open reading frame encodes a protein from the SARS-COV-2 genome. In some embodiments, the open reading frame encodes a SARS-COV-2 Omicron BA.4 and BA.5 receptor-binding domain (RBD). In some embodiments, the open reading frame encoding a SARS-COV-2 BA.4/BA.5 RBD comprises a sequence set forth in SEQ ID NOs: 3-4.

[0176] In some embodiments, the open reading frame encodes a rabies virus (RABV) antigen. In some embodiments, the open reading frame encoding a rabies virus antigen comprises a sequence set forth in SEQ ID NOs: 5-6. In some embodiments, the open reading frame encoding a rabies virus antigen comprises a sequence set forth in SEQ ID NOs: 15-17 or 28-30.

[0177] In some embodiments, the open reading frame encodes a respiratory syncytial virus (RSV) antigen. In some embodiments, the open reading frame encoding a respiratory syncytial virus antigen comprises a sequence set forth in SEQ ID NOs: 7-8. In some

embodiments, the open reading frame encoding a respiratory syncytial virus antigen comprises a sequence set forth in SEQ ID NOs: 9-11 or 22-24.

[0178] In some embodiments, the open reading frame encodes a varicella-zoster virus (VZV) antigen. In some embodiments, the open reading frame encoding a respiratory syncytial virus antigen comprises a sequence set forth in SEQ ID NOs: 12-14 or 25-27.

[0179] In some embodiments, the open reading frame comprises a coding sequence comprising modifications to the ribose backbone and/or the nitrogenous bases. In some embodiments, the coding sequence further comprises at least one modified G, C, or A. In some embodiments, the modified RNA molecule comprises a modified backbone. In some embodiments, the modified backbone comprises at least one phosphorothioate linkage.

[0180] In some embodiments, the present invention provides a method of inducing a mammalian cell to translate a recombinant protein comprising contacting the mammalian cell with an *in vitro* transcribed RNA molecule encoding the recombinant protein thereby producing the recombinant protein in the transfected cell.

V. PHARMACEUTICAL COMPOSITIONS

[0181] In some embodiments, the modified RNA molecule is formulated within a pharmaceutical composition. In some embodiments, the pharmaceutical composition comprises a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutically acceptable carrier comprises any vehicle capable of delivering modified RNA molecules into cells. In some embodiments, the pharmaceutically acceptable carrier comprises a lipid nanoparticle (LNP), a lipoplex, or a liposome. In some embodiments, a lipoplex is a nano-sized lipid based material formed by spontaneous self-assembly of cationic liposomes and nucleic acids. In some embodiments, a liposome is an artificial vesicle having at least one lipid bilayer.

A. Lipid Nanoparticles (LNPs)

[0182] In some embodiments, the pharmaceutical composition comprises a LNP. In some embodiments, the modified RNA is formulated in the LNP, such as those described in International Publication No. WO2012170930, herein incorporated by reference in its entirety. In some embodiments, the particle size of the LNP may be increased and/or decreased. The change in particle size may be able to help counter biological reaction such

as, but not limited to, inflammation or may increase the biological effect of the mRNA when administered to an individual.

[0183] In some embodiments, the LNP comprises between about 30 molar percent to about 55 molar percent of a cationic lipid. In some embodiments, the LNP comprises greater than about 30 molar percent of a cationic lipid, such as greater than any of about 35 molar percent, 40 molar percent, 45 molar percent, 50 molar percent, 55 molar percent, or greater, of a cationic lipid. In some embodiments, the LNP comprises less than about 55 molar percent of a cationic lipid, such as less than any of about 50 molar percent, 45 molar percent, 40 molar percent, 35 molar percent, 30 molar percent, or less, of a cationic lipid.

[0184] In some embodiments, the LNP comprises between about 5 molar percent to about 40 molar percent of a phospholipid. In some embodiments, the LNP comprises greater than about 5 molar percent of a phospholipid, such as greater than any of about 10 molar percent, 15 molar percent, 20 molar percent, 25 molar percent, 30 molar percent, 35 molar percent, 40 molar percent, or greater, of a phospholipid. In some embodiments, the LNP comprises less than about 40 molar percent of a phospholipid, such as less than any of about 35 molar percent, 30 molar percent, 25 molar percent, 20 molar percent, 15 molar percent, 10 molar percent, 5 molar percent, or less, of a phospholipid.

[0185] In some embodiments, the LNP comprises between about 20 molar percent to about 50 molar percent of a sterol. In some embodiments, the LNP comprises greater than about 20 molar percent of a sterol, such as greater than any of about 25 molar percent, 30 molar percent, 35 molar percent, 40 molar percent, 45 molar percent, 50 molar percent, or greater, of a sterol. In some embodiments, the LNP comprises less than about 50 molar percent of a sterol, such as less than any of about 45 molar percent, 40 molar percent, 35 molar percent, 30 molar percent, 25 molar percent, 20 molar percent, or less, of a sterol.

[0186] In some embodiments, the LNP comprises a cationic lipid, a phospholipid, a sterol, and a polymer conjugated lipid, such as any of the cationic lipids, phospholipids, sterols, and polymer conjugated lipids described herein. In some embodiments, the LNP comprises i) between about 30 molar percent to about 55 molar percent of a cationic lipid, ii) between about 5 molar percent to about 40 molar percent of a phospholipid

[0187] In some embodiments, the LNP comprises a total lipid to modified RNA weight ratio of about 10:1 to about 30:1, such as any of about 10:1 to about 20:1, about 15:1 to about 25:1, and about 20:1 to about 30:1. In some embodiments, the LNP comprises a total lipid to modified RNA weight ratio of greater than about 10:1, such as greater than any of about 15:1, 20:1, 25:1, 30:1, or greater. In some embodiments, the LNP comprises a total

lipid to modified RNA weight ratio of less than about 30:1, such as less than any of about 25:1, 20:1, 15:1, 10:1, or less. In some embodiments, the total lipid to mRNA weight ratio may be adjusted depending on the other components of the pharmaceutical composition, the individual to be administered, and/or the route of administration. The amount of mRNA in an LNP, for example, be measured using absorption spectroscopy (e.g., ultraviolet-visible spectroscopy).

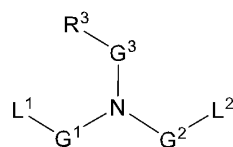
[0188] In some embodiments, the LNPs disclosed herein further comprise a therapeutic payload. The payload can be any substance or compound that has a therapeutic or prophylactic effect. In some embodiments, the therapeutic payload is a small molecule, a cytotoxin, a radioactive ion, a chemotherapeutic compound, a vaccine, or a compound that elicits an immune response.

[0189] In some embodiments, the LNPs disclosed herein comprise a nucleic acid. In some embodiments, the nucleic acid is a DNA. In some embodiments, the DNA is catalytic DNA, plasmid DNA, aptamer, or complementary DNA (cDNA). In some embodiments, the nucleic acid is an RNA. In some embodiments, the RNA is a messenger RNA (mRNA), antisense oligonucleotide, microRNA (miRNA), miRNA inhibitor (e.g., antagomir or antimir), messenger-RNA-interfering complementary RNA (micRNA), multivalent RNA, dicer substrate RNA (dsRNA), small hairpin RNA (shRNA), antisense RNA, transfer RNA (tRNA), asymmetrical interfering RNA (aiRNA), a ribozyme, an aptamer, or a vector. In some embodiments, the RNA is an mRNA hybrid. In some embodiments, the nucleic acid is an mRNA. In some embodiments, the mRNA encodes a protein. In some embodiments, the protein is an antibody. In some embodiments, the antibody is a bispecific antibody. In some embodiments, the LNPs comprise an RNAi agent or RNAi-inducing agent.

B. Cationic Lipids

[0190] In one embodiment, the cationic lipid contained in the compositions, nanoparticle compositions, or nanoparticles described herein is a cationic lipid described in International Patent Publication No. WO2021204175, the entirety of which is incorporated herein by reference.

[0191] In one embodiment, the cationic lipid is a compound of Formula (01-I):



(01-I),

[0192] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof, wherein:

[0193] G1 and G2 are each independently a bond, C2-C12 alkylene, or C2-C12 alkenylene, wherein one or more -CH₂- in the alkylene or alkenylene is optionally replaced by -O-;

[0194] L1 is -OC(=O)R1, -C(=O)OR1, -OC(=O)OR1, -C(=O)R1, -OR1, -S(O)_xR1, -S-SR1, -C(=O)SR1, -SC(=O)R1, -NRaC(=O)R1, -C(=O)NRbRc, -NRaC(=O)NRbRc, -OC(=O)NRbRc, -NRaC(=O)OR1, -SC(=S)R1, -C(=S)SR1, -C(=S)R1, -CH(OH)R1, -P(=O)(ORb)(ORc), -(C6-C10 arylene)-R1, -(6- to 10-membered heteroarylene)-R1, or R1;

[0195] L2 is -OC(=O)R2, -C(=O)OR2, -OC(=O)OR2, -C(=O)R2, -OR2, -S(O)_xR2, -S-SR2, -C(=O)SR2, -SC(=O)R2, -NRdC(=O)R2, -C(=O)NReRf, -NRdC(=O)NReRf, -OC(=O)NReRf, -NRdC(=O)OR2, -SC(=S)R2, -C(=S)SR2, -C(=S)R2, -CH(OH)R2, -P(=O)(ORe)(ORf), -(C6-C10 arylene)-R2, -(6- to 10-membered heteroarylene)-R2, or R2;

[0196] R1 and R2 are each independently C6-C32 alkyl or C6-C32 alkenyl;

[0197] Ra, Rb, Rd, and Re are each independently H, C1-C24 alkyl, or C2-C24 alkenyl;

[0198] Rc and Rf are each independently C1-C32 alkyl or C2-C32 alkenyl;

[0199] G3 is C2-C24 alkylene, C2-C24 alkenylene, C3-C8 cycloalkylene, or C3-C8 cycloalkenylene;

[0200] R3 is -N(R4)R5;

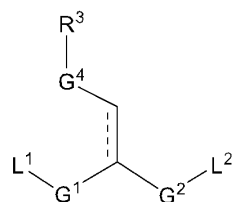
[0201] R4 is C3-C8 cycloalkyl, C3-C8 cycloalkenyl, 4- to 8-membered heterocyclyl, or C6-C10 aryl; or R4, G3 or part of G3, together with the nitrogen to which they are attached form a cyclic moiety;

[0202] R5 is C1-C12 alkyl or C3-C8 cycloalkyl; or R4, R5, together with the nitrogen to which they are attached form a cyclic moiety;

[0203] x is 0, 1 or 2; and

[0204] wherein each alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocyclyl, aryl, alkylene, alkenylene, cycloalkylene, cycloalkenylene, arylene, heteroarylene, and cyclic moiety is independently optionally substituted.

[0205] In one embodiment, the cationic lipid is a compound of Formula (01-II):



(01-II),

[0206] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof, wherein:

[0207] ----- is a single bond or a double bond;

[0208] G1 and G2 are each independently a bond, C2-C12 alkylene, or C2-C12 alkenylene, wherein one or more -CH₂- in the alkylene or alkenylene is optionally replaced by -O-;

[0209] L1 is -OC(=O)R₁, -C(=O)OR₁, -OC(=O)OR₁, -C(=O)R₁, -OR₁, -S(O)_xR₁, -S-SR₁, -C(=O)SR₁, -SC(=O)R₁, -NR_aC(=O)R₁, -C(=O)NR_bR_c, -NR_aC(=O)NR_bR_c, -OC(=O)NR_bR_c, -NR_aC(=O)OR₁, -SC(=S)R₁, -C(=S)SR₁, -C(=S)R₁, -CH(OH)R₁, -P(=O)(OR_b)(OR_c), -(C₆-C₁₀ arylene)-R₁, -(6- to 10-membered heteroarylene)-R₁, or R₁;

[0210] L2 is -OC(=O)R₂, -C(=O)OR₂, -OC(=O)OR₂, -C(=O)R₂, -OR₂, -S(O)_xR₂, -S-SR₂, -C(=O)SR₂, -SC(=O)R₂, -NR_dC(=O)R₂, -C(=O)NR_eR_f, -NR_dC(=O)NR_eR_f, -OC(=O)NR_eR_f, -NR_dC(=O)OR₂, -SC(=S)R₂, -C(=S)SR₂, -C(=S)R₂, -CH(OH)R₂, -P(=O)(OR_e)(OR_f), -(C₆-C₁₀ arylene)-R₂, -(6- to 10-membered heteroarylene)-R₂, or R₂;

[0211] R₁ and R₂ are each independently C₆-C₃₂ alkyl or C₆-C₃₂ alkenyl;

[0212] R_a, R_b, R_d, and R_e are each independently H, C₁-C₂₄ alkyl, or C₂-C₂₄ alkenyl;

[0213] R_c and R_f are each independently C₁-C₃₂ alkyl or C₂-C₃₂ alkenyl;

[0214] G₄ is a bond, C₁-C₂₃ alkylene, C₂-C₂₃ alkenylene, C₃-C₈ cycloalkylene, or C₃-C₈ cycloalkenylene;

[0215] R₃ is -N(R₄)R₅;

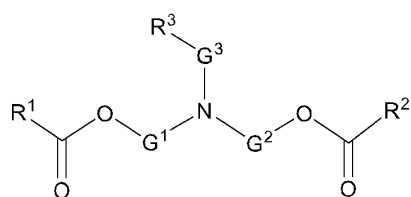
[0216] R₄ is C₁-C₁₂ alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloalkenyl, 4- to 8-membered heterocyclyl, or C₆-C₁₀ aryl; or R₄, G₃ or part of G₃, together with the nitrogen to which they are attached form a cyclic moiety;

[0217] R₅ is C₁-C₁₂ alkyl or C₃-C₈ cycloalkyl; or R₄, R₅, together with the nitrogen to which they are attached form a cyclic moiety;

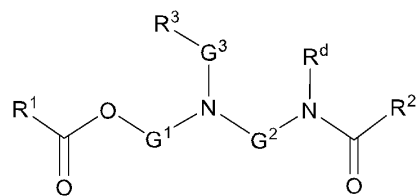
[0218] x is 0, 1 or 2; and

[0219] wherein each alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocyclyl, aryl, alkylene, alkenylene, cycloalkylene, cycloalkenylene, arylene, heteroarylene, and cyclic moiety is independently optionally substituted.

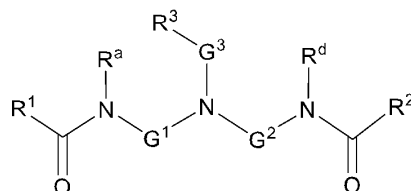
[0220] In one embodiment, the compound is a compound of Formula (01-I-B), (01-I-B'), (01-I-B''), (01-I-C), (01-I-D), or (01-I-E):



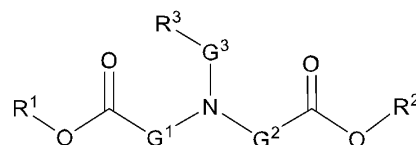
(01-I-B),



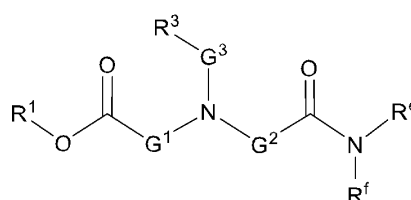
(01-I-B'),



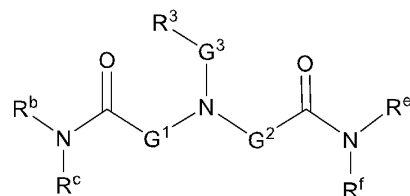
(01-I-B''),



(01-I-C),



(01-I-D), or

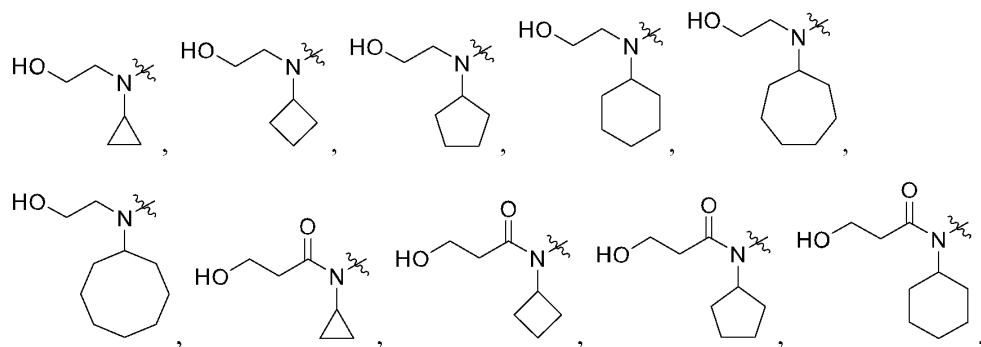


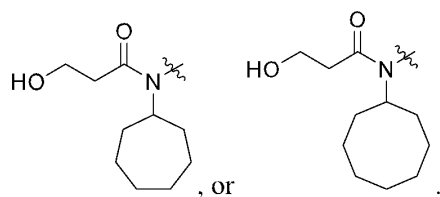
(01-I-E),

[0221] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof.

[0222] In one embodiment, G1 and G2 are each independently C3-C7 alkylene. In one embodiment, G1 and G2 are each independently C5 alkylene. In one embodiment, G3 is C2-C4 alkylene. In one embodiment, G3 is C2 alkylene. In one embodiment, G3 is C4 alkylene.

[0223] In one embodiment, R3 has one of the following structures:



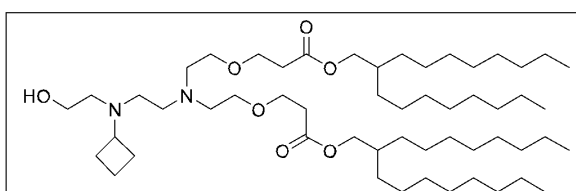


[0224] In one embodiment, R1, R2, Rc and Rf are each independently branched C6-C32 alkyl or branched C6-C32 alkenyl. In one embodiment, R1, R2, Rc and Rf are each independently branched C6-C24 alkyl or branched C6-C24 alkenyl. In one embodiment, R1, R2, Rc and Rf are each independently -R7-CH(R8)(R9), wherein R7 is C0-C5 alkylene, and R8 and R9 are independently C2-C10 alkyl. In one embodiment, R1, R2, Rc and Rf are each independently -R7-CH(R8)(R9), wherein R7 is C0-C1 alkylene, and R8 and R9 are independently C4-C8 alkyl.

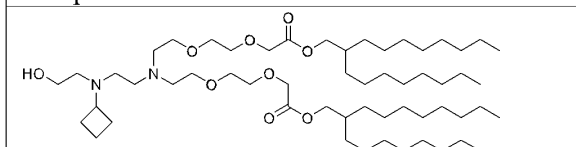
[0225] In one embodiment, the compound is a compound in Table 1, or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof.

Table 1:

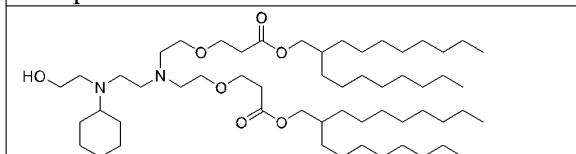
Compound 01-1
Compound 01-2
Compound 01-20
Compound 01-21



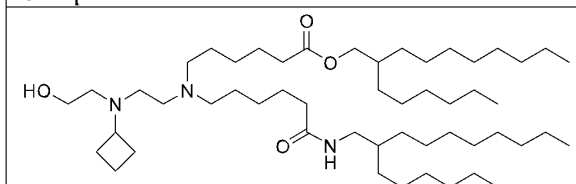
Compound 01-102



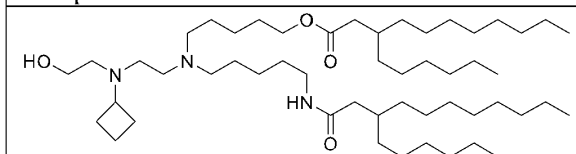
Compound 01-103



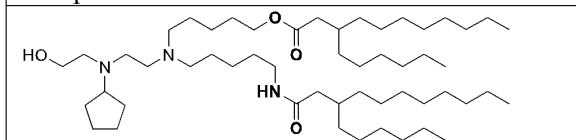
Compound 01-104



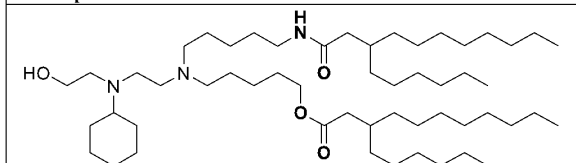
Compound 01-106



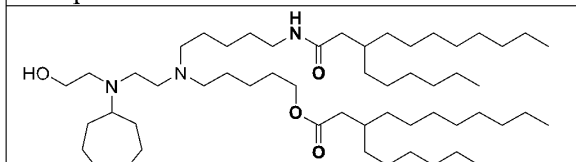
Compound 01-108



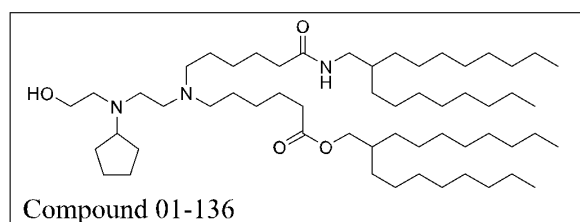
Compound 01-130



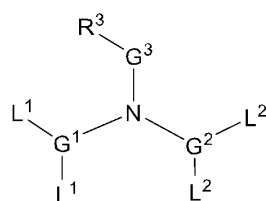
Compound 01-131



Compound 01-132



[0226] In one embodiment, the cationic lipid contained in the compositions, nanoparticle compositions, or nanoparticles provided herein is a cationic lipid described in International Patent Publication No. WO 2023/138611, the entirety of which is incorporated herein by reference. In one embodiment, the cationic lipid is a compound of Formula (02-I):



(02-I),

[0227] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof, wherein:

[0228] G1 and G2 are each independently C2-C12 alkylene or C2-C12 alkenylene, wherein one or more -CH2- in G1 and G2 is optionally replaced by -O-, -C(=O)O-, or -OC(=O)-;

[0229] each L1 is independently -OC(=O)R1, -C(=O)OR1, -OC(=O)OR1, -C(=O)R1, -OR1, -S(O)_xR1, -S-SR1, -C(=O)SR1, -SC(=O)R1, -NRaC(=O)R1, -C(=O)NRbRc, -NRaC(=O)NRbRc, -OC(=O)NRbRc, -NRaC(=O)OR1, -SC(=S)R1, -C(=S)SR1, -C(=S)R1, -CH(OH)R1, -P(=O)(ORb)(ORc), -NRaP(=O)(ORb)(ORc);

[0230] each L2 is independently -OC(=O)R2, -C(=O)OR2, -OC(=O)OR2, -C(=O)R2, -OR2, -S(O)_xR2, -S-SR2, -C(=O)SR2, -SC(=O)R2, -NRdC(=O)R2, -C(=O)NReRf, -NRdC(=O)NReRf, -OC(=O)NReRf, -NRdC(=O)OR2, -SC(=S)R2, -C(=S)SR2, -C(=S)R2, -CH(OH)R2, -P(=O)(ORe)(ORf), -NRdP(=O)(ORe)(ORf);

[0231] R1 and R2 are each independently C6-C24 alkyl or C6-C24 alkenyl;

[0232] Ra, Rb, Rd, and Re are each independently H, C1-C24 alkyl, or C2-C24 alkenyl;

[0233] Rc and Rf are each independently C1-C24 alkyl or C2-C24 alkenyl;

[0234] G3 is C2-C12 alkylene or C2-C12 alkenylene, wherein part or all of alkylene or alkenylene is optionally replaced by a C3-C8 cycloalkylene or C3-C8 cycloalkenylene;

[0235] R3 is -N(R4)R5, -OR6, or -SR6;

[0236] R₄ is C1-C12 alkyl, C2-C12 alkenyl, C3-C8 cycloalkyl, C3-C8 cycloalkenyl, C6-C10 aryl, or 4- to 8-membered heterocycloalkyl;

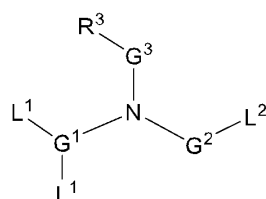
[0237] R₅ is H, C1-C12 alkyl, C3-C8 cycloalkyl, C3-C8 cycloalkenyl, C6-C10 aryl, or 4- to 8-membered heterocycloalkyl;

[0238] R₆ is hydrogen, C1-C12 alkyl, C3-C8 cycloalkyl, C3-C8 cycloalkenyl, or C6-C10 aryl;

[0239] x is 0, 1, or 2; and

[0240] wherein each alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, alkylene, alkenylene, cycloalkylene, and cycloalkenylene is independently optionally substituted.

[0241] In one embodiment, the cationic lipid is a compound of Formula (02-II):



(02-II),

[0242] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof, wherein:

[0243] G₁ and G₂ are each independently C2-C12 alkylene or C2-C12 alkenylene, wherein one or more -CH₂- in G₁ and G₂ is optionally replaced by -O-, -C(=O)O-, or -OC(=O)-;

[0244] each L₁ is independently -OC(=O)R₁, -C(=O)OR₁, -OC(=O)OR₁, -C(=O)R₁, -OR₁, -S(O)_xR₁, -S-SR₁, -C(=O)SR₁, -SC(=O)R₁, -NRaC(=O)R₁, -C(=O)NRbRc, -NRaC(=O)NRbRc, -OC(=O)NRbRc, -NRaC(=O)OR₁, -SC(=S)R₁, -C(=S)SR₁, -C(=S)R₁, -CH(OH)R₁, -P(=O)(ORb)(ORc), -NRaP(=O)(ORb)(ORc);

[0245] each L₂ is independently -OC(=O)R₂, -C(=O)OR₂, -OC(=O)OR₂, -C(=O)R₂, -OR₂, -S(O)_xR₂, -S-SR₂, -C(=O)SR₂, -SC(=O)R₂, -NRdC(=O)R₂, -C(=O)NRf, -NRdC(=O)NRf, -OC(=O)NRf, -NRdC(=O)OR₂, -SC(=S)R₂, -C(=S)SR₂, -C(=S)R₂, -CH(OH)R₂, -P(=O)(ORe)(ORf), -NRdP(=O)(ORe)(ORf);

[0246] R₁ and R₂ are each independently C6-C24 alkyl or C6-C24 alkenyl;

[0247] Ra, Rb, Rd, and Re are each independently H, C1-C24 alkyl, or C2-C24 alkenyl;

[0248] Rc and Rf are each independently C1-C24 alkyl or C2-C24 alkenyl;

[0249] G₃ is C2-C12 alkylene or C2-C12 alkenylene, wherein part or all of alkylene or alkenylene is optionally replaced by a C3-C8 cycloalkylene or C3-C8 cycloalkenylene;

[0250] R³ is -N(R⁴)R⁵, -OR⁶, or -SR⁶;

[0251] R⁴ is C1-C12 alkyl, C2-C12 alkenyl, C3-C8 cycloalkyl, C3-C8 cycloalkenyl, C6-C10 aryl, or 4- to 8-membered heterocycloalkyl;

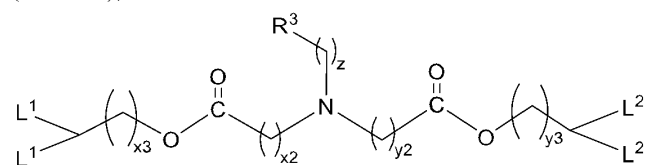
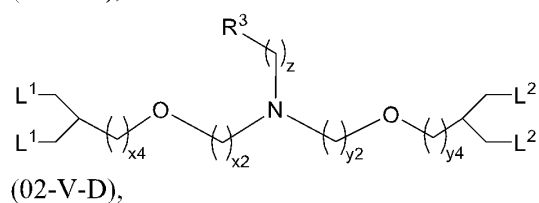
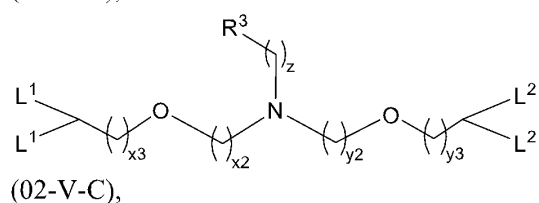
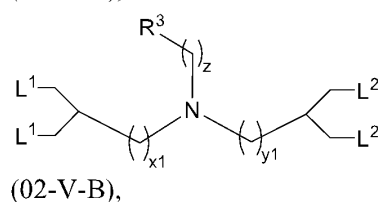
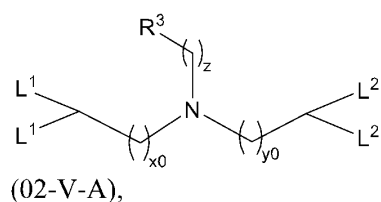
[0252] R⁵ is H, C1-C12 alkyl, C3-C8 cycloalkyl, C3-C8 cycloalkenyl, C6-C10 aryl, or 4- to 8-membered heterocycloalkyl;

[0253] R⁶ is hydrogen, C1-C12 alkyl, C3-C8 cycloalkyl, C3-C8 cycloalkenyl, or C6-C10 aryl;

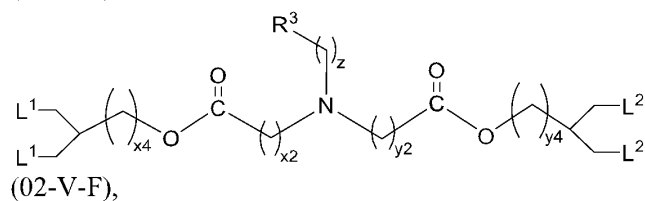
[0254] x is 0, 1, or 2; and

[0255] wherein each alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, alkylene, alkenylene, cycloalkylene, and cycloalkenylene is independently optionally substituted.

[0256] In one embodiment, the compound is a compound of Formula (02-V-A), (02-V-B), (02-V-C), (02-V-D), (02-V-E), (02-V-F):



(02-V-E), or



[0257] wherein z is an integer from 2 to 12,

[0258] x0 is an integer from 1 to 11;

[0259] y0 is an integer from 1 to 11;

[0260] x1 is an integer from 0 to 9;

[0261] y1 is an integer from 0 to 9;

[0262] x2 is an integer from 2 to 5;

[0263] x3 is an integer from 1 to 5;

[0264] x4 is an integer from 0 to 3;

[0265] y2 is an integer from 2 to 5;

[0266] y3 is an integer from 1 to 5; and

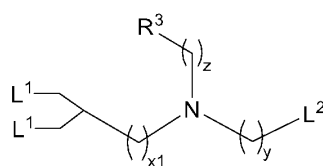
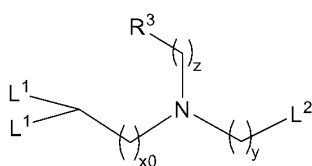
[0267] y4 is an integer from 0 to 3;

[0268] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof.

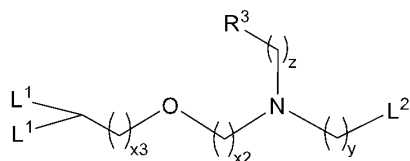
[0269] In one embodiment, z is an integer from 2 to 6. In one embodiment, z is 2, 4, or 5. In one embodiment, x0 and y0 are independently 2 to 6. In one embodiment, x0 and y0 are independently 4 or 5. In one embodiment, x1 and y1 are independently 2 to 6. In one embodiment, x1 and y1 are independently 4 or 5. In one embodiment, x2 and y2 are independently an integer from 2 to 5. In one embodiment, x2 and y2 are independently 3 or 5. In one embodiment, x3 and y3 are both 1. In one embodiment, x4 and y4 are independently 0 or 1.

[0270] In one embodiment, each L1 is independently -OR1, -OC(=O)R1 or -C(=O)OR1, and each L2 is independently -OR2, -OC(=O)R2 or -C(=O)OR2. In one embodiment, R1 and R2 are independently straight C6-C10 alkyl, or -R7-CH(R8)(R9), wherein R7 is C0-C5 alkylene, and R8 and R9 are independently C2-C10 alkyl or C2-C10 alkenyl.

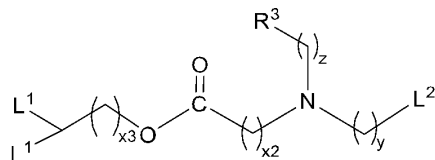
[0271] In one embodiment, the compound is a compound of formula (02-VI-A), (02-VI-B), (02-VI-C), (02-VI-D), (02-VI-E), or (02-VI-F):



(02-VI-A),



(02-VI-C),



(02-VI-E), or

[0272] wherein z is an integer from 2 to 12;

[0273] y is an integer from 2 to 12;

[0274] x0 is an integer from 1 to 11;

[0275] x1 is an integer from 0 to 9;

[0276] x2 is an integer from 2 to 5;

[0277] x3 is an integer from 1 to 5; and

[0278] x4 is an integer from 0 to 3;

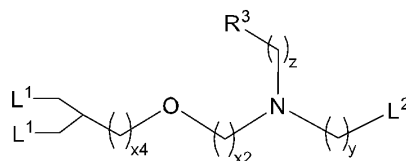
[0279] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof.

[0280] In one embodiment, z is an integer from 2 to 6. In one embodiment, z is 2, 4 or 5. In one embodiment, x0 is 4 or 5. In one embodiment, x1 is 4 or 5. In one embodiment, x2 is an integer from 2 to 5. In one embodiment, x2 is 3 or 5. In one embodiment, x3 is 0 or 1. In one embodiment, y is an integer from 2 to 6. In one embodiment, y is 5.

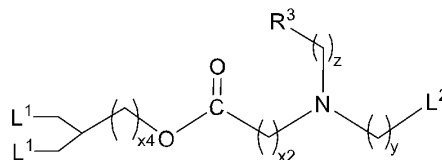
[0281] In one embodiment, each L1 is independently -OR1, -OC(=O)R1 or -C(=O)OR1, and L2 is -OC(=O)R2 or -C(=O)OR2, -NRdC(=O)R2, or -C(=O)NRdRf. In one embodiment, R1 is straight C6-C10 alkyl or -R7-CH(R8)(R9), wherein R7 is C0-C5 alkylene, and R8 and R9 are independently C2-C10 alkyl or C2-C10 alkenyl. In one embodiment, R2 and Rf are each independently straight C6-C18 alkyl, C6-C18 alkenyl, or -R7-CH(R8)(R9), wherein R7 is C0-C5 alkylene, and R8 and R9 are independently C2-C10 alkyl or C2-C10 alkenyl. In one embodiment, Rd and Re are each independently H.

[0282] In one embodiment, the compound is a compound in Table 2, or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof.

(02-VI-B),

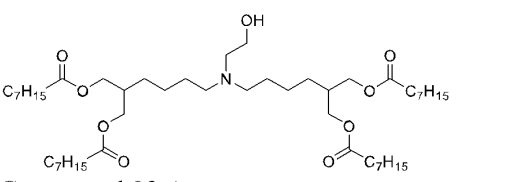
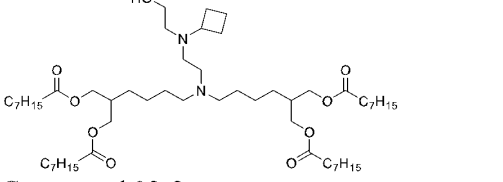
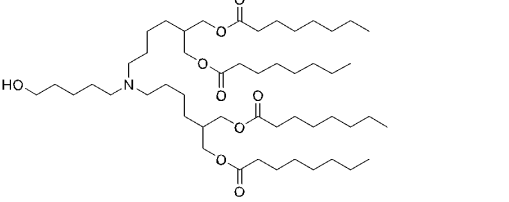
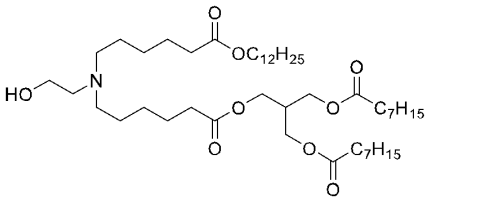
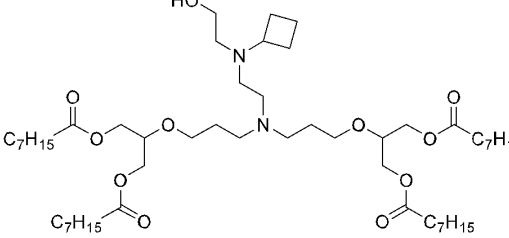
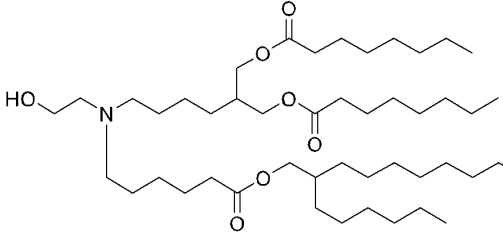
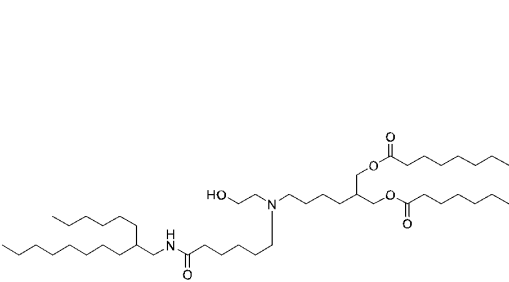
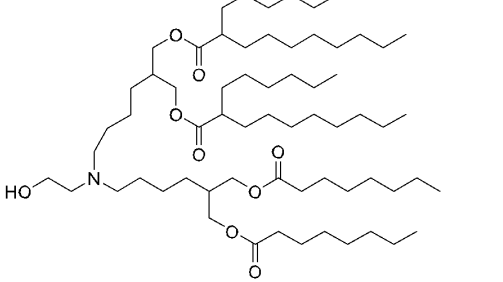
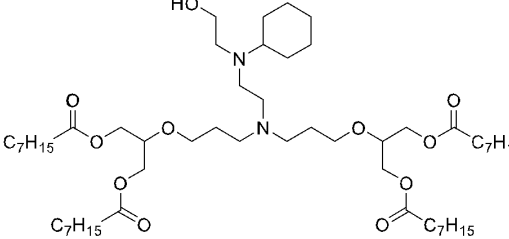
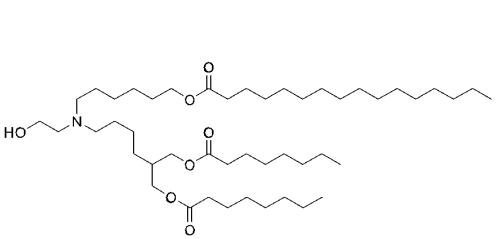
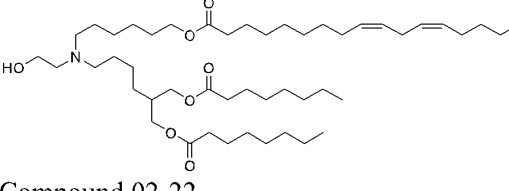


(02-VI-D),



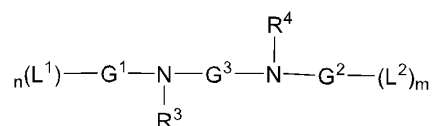
(02-VI-F),

Table 2:

 Compound 02-1	 Compound 02-2
 Compound 02-3	 Compound 02-4
 Compound 02-9	 Compound 02-10
 Compound 02-11	 Compound 02-12
 Compound 02-14	 Compound 02-20
 Compound 02-22	

[0283] In one embodiment, the cationic lipid contained in the compositions, nanoparticle compositions, or nanoparticles described herein is a cationic lipid described in International Patent Publication No. WO2022152109, the entirety of which is incorporated herein by reference.

[0284] In one embodiment, the cationic lipid is a compound of Formula (03-I):



(03-I),

[0285] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof, wherein:

[0286] G1 and G2 are each independently a bond, C2-C12 alkylene, or C2-C12 alkenylene, wherein one or more -CH2- in G1 and G2 is optionally replaced by -O-;

[0287] each L1 is independently -OC(=O)R1, -C(=O)OR1, -OC(=O)OR1, -C(=O)R1, -OR1, -S(O)_xR1, -S-SR1, -C(=O)SR1, -SC(=O)R1, -NRaC(=O)R1, -C(=O)NRbRc, -NRaC(=O)NRbRc, -OC(=O)NRbRc, -NRaC(=O)OR1, -SC(=S)R1, -C(=S)SR1, -C(=S)R1, -CH(OH)R1, -P(=O)(ORb)(ORc), -NRaP(=O)(ORb)(ORc), -(C6-C10 arylene)-R1, -(6- to 10-membered heteroarylene)-R1, -(4- to 8-membered heterocyclylene)-R1, or R1;

[0288] each L2 is independently -OC(=O)R2, -C(=O)OR2, -OC(=O)OR2, -C(=O)R2, -OR2, -S(O)_xR2, -S-SR2, -C(=O)SR2, -SC(=O)R2, -NRdC(=O)R2, -C(=O)NReRf, -NRdC(=O)NReRf, -OC(=O)NReRf, -NRdC(=O)OR2, -SC(=S)R2, -C(=S)SR2, -C(=S)R2, -CH(OH)R2, -P(=O)(ORe)(ORf), -NRdP(=O)(ORe)(ORf), -(C6-C10 arylene)-R2, -(6- to 10-membered heteroarylene)-R2, -(4- to 8-membered heterocyclylene)-R2, or R2;

[0289] R1 and R2 are each independently C6-C24 alkyl or C6-C24 alkenyl;

[0290] Ra, Rb, Rd, and Re are each independently H, C1-C24 alkyl, or C2-C24 alkenyl;

[0291] Rc and Rf are each independently C1-C24 alkyl or C2-C24 alkenyl;

[0292] G3 is C2-C12 alkylene or C2-C12 alkenylene, wherein part or all of alkylene or alkenylene is optionally replaced by C3-C8 cycloalkylene, C3-C8 cycloalkenylene, C3-C8 cycloalkynylene, 4- to 8-membered heterocyclylene, C6-C10 arylene, or 5- to 10-membered heteroarylene;

[0293] R3 is hydrogen, C1-C12 alkyl, C2-C12 alkenyl, C2-C12 alkynyl, C3-C8 cycloalkyl, C3-C8 cycloalkenyl, C3-C8 cycloalkynyl, 4- to 8-membered heterocyclyl, C6-C10 aryl, or 5- to 10-membered heteroaryl; or R3, G1 or part of G1, together with the

nitrogen to which they are attached form a cyclic moiety; or R₃, G₃ or part of G₃, together with the nitrogen to which they are attached form a cyclic moiety;

[0294] R₄ is C₁-C₁₂ alkyl or C₃-C₈ cycloalkyl;

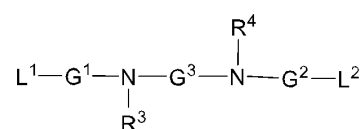
[0295] x is 0, 1, or 2;

[0296] n is 1 or 2;

[0297] m is 1 or 2; and

[0298] wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocyclyl, aryl, heteroaryl, alkylene, alkenylene, cycloalkylene, cycloalkenylene, cycloalkynylene, heterocyclylene, arylene, heteroarylene, and cyclic moiety is independently optionally substituted.

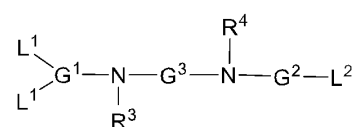
[0299] In one embodiment, the compound is a compound of Formula (03-II-A):



(03-II-A),

[0300] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof.

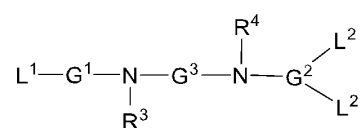
[0301] In one embodiment, the compound is a compound of Formula (03-II-B):



(03-II-B),

[0302] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof.

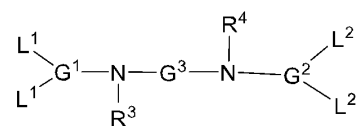
[0303] In one embodiment, the compound is a compound of Formula (03-II-C):



(03-II-C),

[0304] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof.

[0305] In one embodiment, the compound is a compound of Formula (03-II-D):



(03-II-D),

[0306] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof.

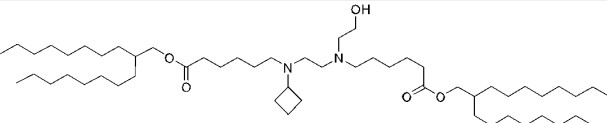
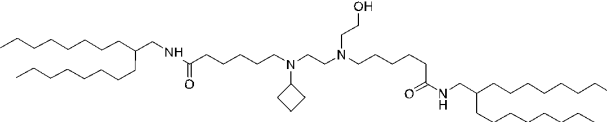
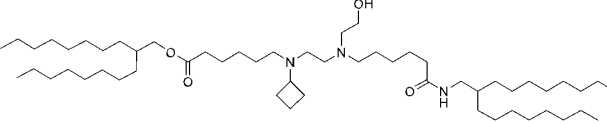
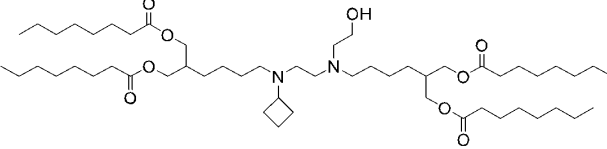
[0307] In one embodiment, G1 and G2 are each independently C2-C12 alkylene. In one embodiment, G1 and G2 are each independently C5 alkylene. In one embodiment, G3 is C2-C6 alkylene.

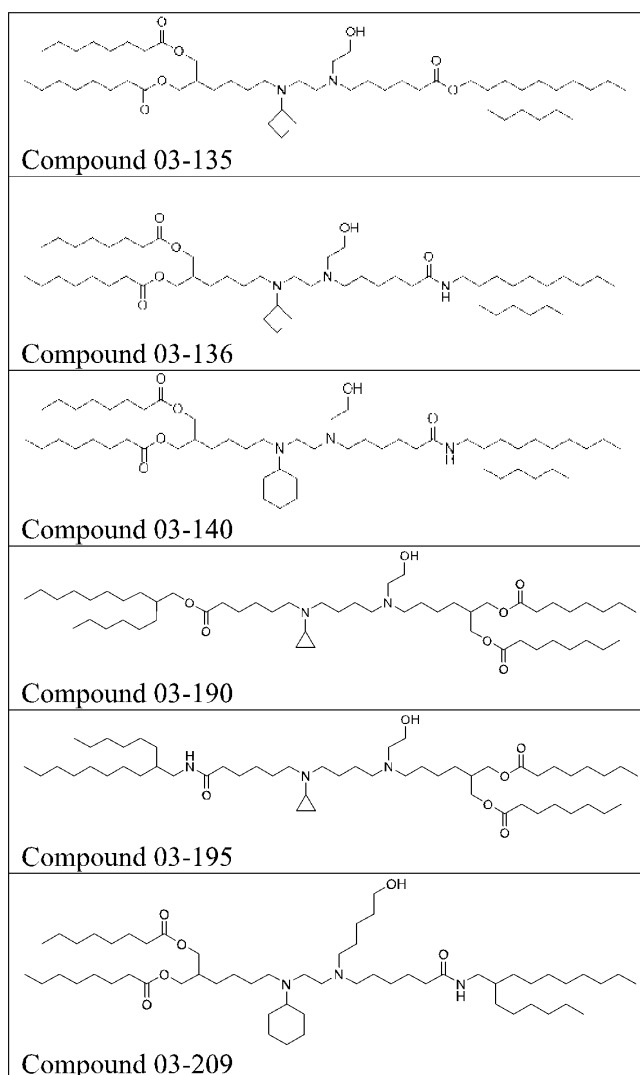
[0308] In one embodiment, R3 is C1-C12 alkyl, C2-C12 alkenyl, or C3-C8 cycloalkyl. In one embodiment, R3 is C3-C8 cycloalkyl. In one embodiment, R3 is unsubstituted. In one embodiment, R4 is substituted C1-C12 alkyl. In one embodiment, R4 is $-\text{CH}_2\text{CH}_2\text{OH}$.

[0309] In one embodiment, L1 is $-\text{OC}(=\text{O})\text{R}_1$, $-\text{C}(=\text{O})\text{OR}_1$, $-\text{NRaC}(=\text{O})\text{R}_1$, or $-\text{C}(=\text{O})\text{NRbRc}$; and L2 is $-\text{OC}(=\text{O})\text{R}_2$, $-\text{C}(=\text{O})\text{OR}_2$, $-\text{NRdC}(=\text{O})\text{R}_2$, or $-\text{C}(=\text{O})\text{NRfRe}$. In one embodiment, R1, R2, Rc, and Rf are each independently straight C6-C18 alkyl, straight C6-C18 alkenyl, or $-\text{R}_7\text{-CH}(\text{R}_8)(\text{R}_9)$, wherein R7 is C0-C5 alkylene, and R8 and R9 are independently C2-C10 alkyl or C2-C10 alkenyl. In one embodiment, R1, R2, Rc, and Rf are each independently straight C7-C15 alkyl, straight C7-C15 alkenyl, or $-\text{R}_7\text{-CH}(\text{R}_8)(\text{R}_9)$, wherein R7 is C0-C1 alkylene, and R8 and R9 are independently C4-C8 alkyl or C6-C10 alkenyl. In one embodiment, Ra, Rb, Rd, and Re are each independently H.

[0310] In one embodiment, the compound is a compound in Table 3, or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof.

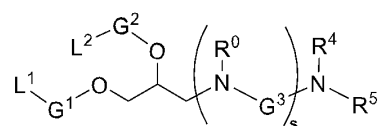
Table 3:


Compound 03-10

Compound 03-11

Compound 03-15

Compound 03-71



[0311] In one embodiment, the cationic lipid contained in the particles or compositions provided herein is a cationic lipid described in International Patent Application No. WO2022247755A1, the entirety of which is incorporated herein by reference.

[0312] In one embodiment, the cationic lipid is a compound of Formula (04-I):



(04-I),

[0313] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof, wherein:

[0314] G¹ and G² are each independently a bond, C₂-C₁₂ alkylene, or C₂-C₁₂ alkenylene;

[0315] L1 is $-\text{OC}(=\text{O})\text{R}_1$, $-\text{C}(=\text{O})\text{OR}_1$, $-\text{OC}(=\text{O})\text{OR}_1$, $-\text{C}(=\text{O})\text{R}_1$, $-\text{OR}_1$, $-\text{S}(\text{O})_x\text{R}_1$, $-\text{S}-\text{SR}_1$, $-\text{C}(=\text{O})\text{SR}_1$, $-\text{SC}(=\text{O})\text{R}_1$, $-\text{NRaC}(=\text{O})\text{R}_1$, $-\text{C}(=\text{O})\text{NRbRc}$, $-\text{NRaC}(=\text{O})\text{NRbRc}$, $-\text{OC}(=\text{O})\text{NRbRc}$, $-\text{NRaC}(=\text{O})\text{OR}_1$, $-\text{SC}(=\text{S})\text{R}_1$, $-\text{C}(=\text{S})\text{SR}_1$, $-\text{C}(=\text{S})\text{R}_1$, $-\text{CH}(\text{OH})\text{R}_1$, $-\text{P}(=\text{O})(\text{ORb})(\text{ORc})$, $-(\text{C}_6\text{-C}_{10}\text{ arylene})-\text{R}_1$, $-(6\text{- to }10\text{-membered heteroarylene})-\text{R}_1$, or R_1 ;

[0316] L2 is $-\text{OC}(=\text{O})\text{R}_2$, $-\text{C}(=\text{O})\text{OR}_2$, $-\text{OC}(=\text{O})\text{OR}_2$, $-\text{C}(=\text{O})\text{R}_2$, $-\text{OR}_2$, $-\text{S}(\text{O})_x\text{R}_2$, $-\text{S}-\text{SR}_2$, $-\text{C}(=\text{O})\text{SR}_2$, $-\text{SC}(=\text{O})\text{R}_2$, $-\text{NRdC}(=\text{O})\text{R}_2$, $-\text{C}(=\text{O})\text{NReRf}$, $-\text{NRdC}(=\text{O})\text{NReRf}$, $-\text{OC}(=\text{O})\text{NReRf}$, $-\text{NRdC}(=\text{O})\text{OR}_2$, $-\text{SC}(=\text{S})\text{R}_2$, $-\text{C}(=\text{S})\text{SR}_2$, $-\text{C}(=\text{S})\text{R}_2$, $-\text{CH}(\text{OH})\text{R}_2$, $-\text{P}(=\text{O})(\text{ORe})(\text{ORf})$, $-(\text{C}_6\text{-C}_{10}\text{ arylene})-\text{R}_2$, $-(6\text{- to }10\text{-membered heteroarylene})-\text{R}_2$, or R_2 ;

[0317] R_1 and R_2 are each independently $\text{C}_5\text{-C}_{32}$ alkyl or $\text{C}_5\text{-C}_{32}$ alkenyl;

[0318] Ra , Rb , Rd , and Re are each independently H , $\text{C}_1\text{-C}_{24}$ alkyl, or $\text{C}_2\text{-C}_{24}$ alkenyl;

[0319] Rc and Rf are each independently $\text{C}_1\text{-C}_{32}$ alkyl or $\text{C}_2\text{-C}_{32}$ alkenyl;

[0320] R_0 is $\text{C}_1\text{-C}_{12}$ alkyl, $\text{C}_2\text{-C}_{12}$ alkenyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_3\text{-C}_8$ cycloalkenyl, $\text{C}_6\text{-C}_{10}$ aryl, or 4- to 8-membered heterocycloalkyl;

[0321] G_3 is $\text{C}_2\text{-C}_{12}$ alkylene or $\text{C}_2\text{-C}_{12}$ alkenylene;

[0322] R_4 is $\text{C}_1\text{-C}_{12}$ alkyl, $\text{C}_2\text{-C}_{12}$ alkenyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_3\text{-C}_8$ cycloalkenyl, $\text{C}_6\text{-C}_{10}$ aryl, or 4- to 8-membered heterocycloalkyl;

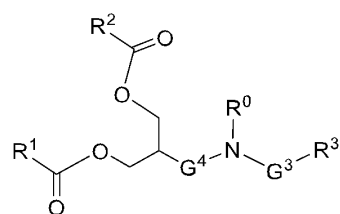
[0323] R_5 is $\text{C}_1\text{-C}_{12}$ alkyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_3\text{-C}_8$ cycloalkenyl, $\text{C}_6\text{-C}_{10}$ aryl, or 4- to 8-membered heterocycloalkyl;

[0324] x is 0, 1, or 2;

[0325] s is 0 or 1; and

[0326] wherein each alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, alkylene, alkenylene, arylene, and heteroarylene, is independently optionally substituted.

[0327] In one embodiment, the cationic lipid is a compound of Formula (04-III):



(04-III),

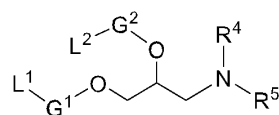
[0328] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof, wherein:

[0329] R_1 and R_2 are each independently $\text{C}_5\text{-C}_{32}$ alkyl or $\text{C}_5\text{-C}_{32}$ alkenyl;

[0330] R_0 is $\text{C}_1\text{-C}_{12}$ alkyl, $\text{C}_2\text{-C}_{12}$ alkenyl, $\text{C}_3\text{-C}_8$ cycloalkyl, $\text{C}_3\text{-C}_8$ cycloalkenyl, $\text{C}_6\text{-C}_{10}$ aryl, or 4- to 8-membered heterocycloalkyl;

[0331] G_3 is $\text{C}_2\text{-C}_{12}$ alkylene or $\text{C}_2\text{-C}_{12}$ alkenylene;

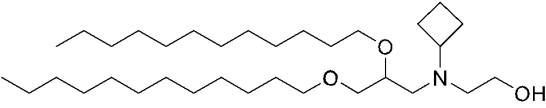
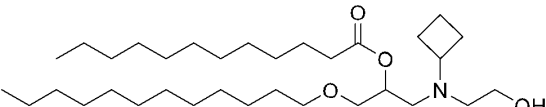
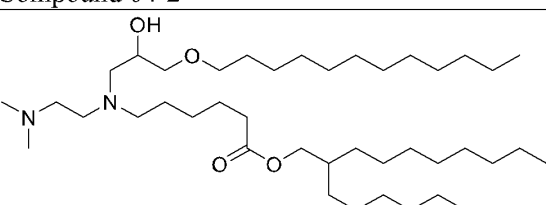
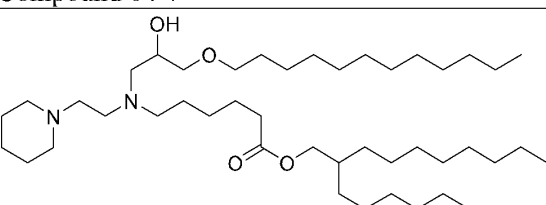
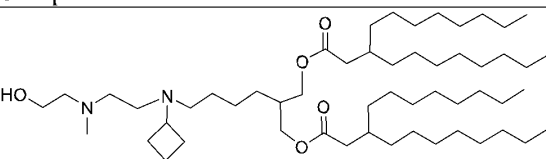
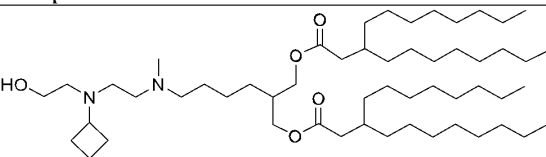
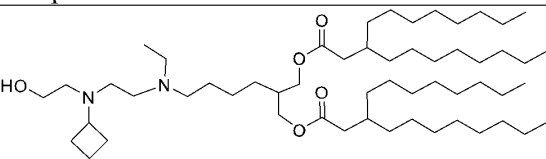
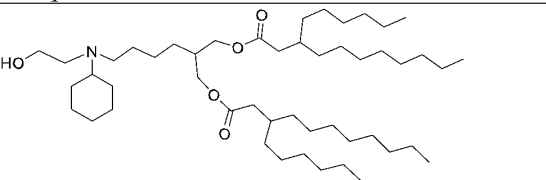
- [0332] G4 is C2-C12 alkylene or C2-C12 alkenylene;
- [0333] R3 is -N(R4)R5 or -OR6;
- [0334] R4 is C1-C12 alkyl, C2-C12 alkenyl, C3-C8 cycloalkyl, C3-C8 cycloalkenyl, C6-C10 aryl, or 4- to 8-membered heterocycloalkyl;
- [0335] R5 is C1-C12 alkyl, C3-C8 cycloalkyl, C3-C8 cycloalkenyl, C6-C10 aryl, or 4- to 8-membered heterocycloalkyl; or R4, R5, together with the nitrogen to which they are attached form a cyclic moiety;
- [0336] R6 is hydrogen, C1-C12 alkyl, C3-C8 cycloalkyl, C3-C8 cycloalkenyl, or C6-C10 aryl; and
- [0337] wherein each alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, alkylene, alkenylene, and cyclic moiety is independently optionally substituted.
- [0338] In one embodiment, the compound is a compound of Formula (04-IV):

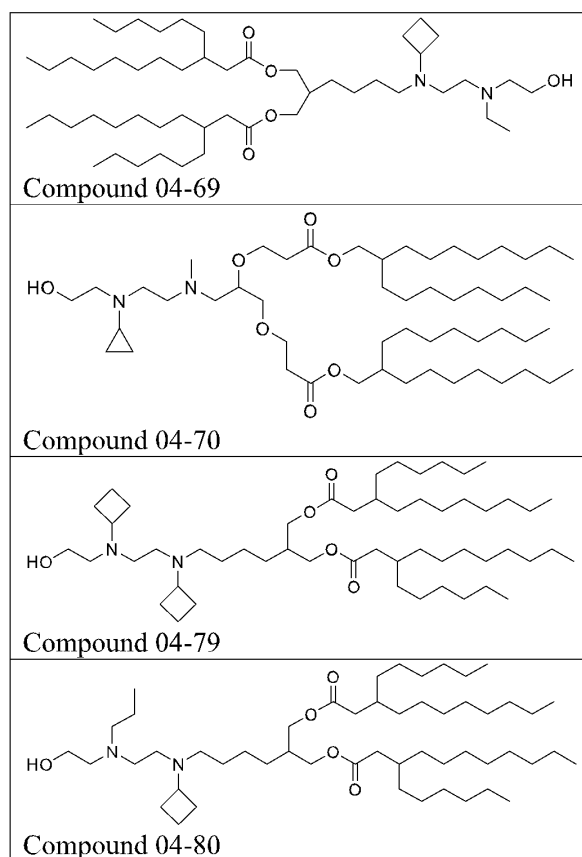


(04-IV),

- [0339] or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof.
- [0340] In one embodiment, G3 is C2-C4 alkylene. In one embodiment, G4 is C2-C4 alkylene.
- [0341] In one embodiment, R0 is C1-C6 alkyl. In one embodiment, R3 is -OH. In one embodiment, R3 is -N(R4)R5. In one embodiment, R4 is C3-C8 cycloalkyl. In one embodiment, R4 is unsubstituted. In one embodiment, R5 is -CH₂CH₂OH.
- [0342] In one embodiment, L1 is -OC(=O)R1, -C(=O)OR1, -C(=O)R1, -C(=O)NRbRc, or R1; and L2 is -OC(=O)R2, -C(=O)OR2, -C(=O)R2, -C(=O)NReRf, or R2. In one embodiment, R1 and R2 are each independently branched C6-C24 alkyl or branched C6-C24 alkenyl. In one embodiment, R1 and R2 are each independently -R7-CH(R8)(R9), wherein R7 is C1-C5 alkylene, and R8 and R9 are independently C2-C10 alkyl or C2-C10 alkenyl. In one embodiment, R1 is straight C6-C24 alkyl and R2 is branched C6-C24 alkyl. In one embodiment, R1 is straight C6-C24 alkyl and R2 is -R7-CH(R8)(R9), wherein R7 is C1-C5 alkylene, and R8 and R9 are independently C2-C10 alkyl.
- [0343] In one embodiment, the compound is a compound in Table 4, or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof.

Table 4:

 Compound 04-1
 Compound 04-2
 Compound 04-7
 Compound 04-8
 Compound 04-65
 Compound 04-66
 Compound 04-67
 Compound 04-68



C. Kits

[0344] The present disclosure includes kits comprising the components for *in vitro* transcription of the modified RNAs disclosed herein. In some embodiments, the kit comprises 1) a DNA-dependent RNA polymerase; 2) modified and/or unmodified nucleotides (for example, modified and/or unmodified adenosines, guanosines, cytidines or uridines), and 3) a 5' cap, and 4) instructions for carrying out the method disclosed herein. In some embodiments, the kit further comprises a 5' cap, wherein the 5' cap has the sequence of N1pppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine. In some embodiments, the kit further comprises a 5' cap, wherein the 5' cap has the sequence, m⁷GpppApU, wherein A is modified or unmodified. In some embodiments, the kit further comprises a 5' cap, wherein the 5' cap has the sequence, m⁷GpppApU, wherein the A is 2'-O-methylated. In some embodiments, the kit further comprises a 5' cap, wherein the 5' cap has the sequence of m⁷GpppA_m. In some embodiments, the kit further comprises a 5' cap, wherein the 5' cap has the sequence of

N1vpppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine. In some embodiments, the kit further comprises a 5' cap, wherein the 5' cap has the sequence, m⁷GvpppApU, wherein A is modified or unmodified. In some embodiments, the kit further comprises a 5' cap, wherein the 5' cap has the sequence, m⁷GvpppApU, wherein the A is 2'-O-methylated. In some embodiments, the kit further comprises a 5' cap, wherein the 5' cap has the sequence of m⁷GvpppA_{mp}U.

[0345] In some embodiments, the kit further comprises modified nucleotides. In some embodiments, the kit further comprises at least one modified G, C, or A. In some embodiments, the kit further comprises a modified uridine. In some embodiments, the kit further comprises a modified uridine, wherein the modified uridine comprises Ψ. In some embodiments, the kit further comprises a modified uridine, wherein the modified uridine comprises m1Ψ.

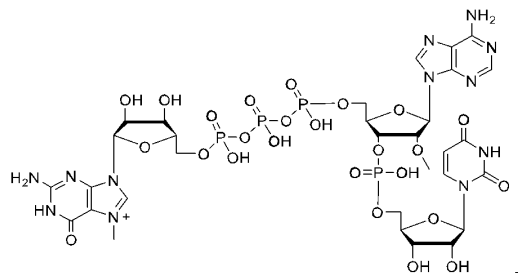
VI. METHODS OF LOWING THE INNATE IMMUNE STIMULATION

[0346] Provided herein are methods of lowering the innate immune stimulation of a RNA molecule.

[0347] In some embodiments, described herein is a method of lowering the innate immune stimulation of a RNA molecule, comprising using a 5' cap having the sequence of N1pppN2pU and modifying at least one uridine in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine.

[0348] In some embodiments, the 5' cap has the sequence of GpppApU, wherein A is modified or unmodified. In some embodiments, the A is 2'-O-methylated. In some embodiments, the 5' cap has the sequence of m⁷GpppApU. In some embodiments, the 5' cap has the sequence of m⁷GpppApU, wherein the A is modified or unmodified. In some embodiments, the A is 2'-O-methylated. In some embodiments, the 5' cap has the sequence of m⁷GpppA_{mp}U. In some embodiments, the 5' cap comprises a nucleic acid sequence set forth in any one of SEQ ID NOs: 2, 4, 6, or 8. In some embodiments, the 5' cap comprise a nucleic acid sequence set forth in any one of SEQ ID NOs: 9, 12, 15, 18, 22, 25, 28 or 31. In some embodiments, the 5' cap comprise a nucleic acid sequence set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 9, 12, 15, 18, 22, 25, 28 or 31.

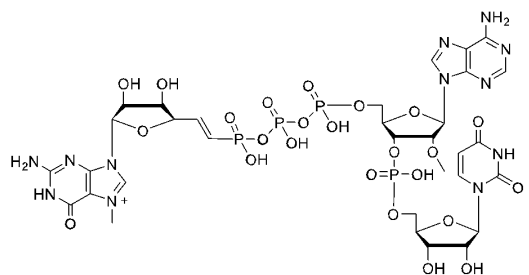
[0349] In some embodiments, $m^7GpppA_{mp}U$ has the following formula,



[0350] In some embodiments, described herein is a method of lowering the innate immune stimulation of a RNA molecule, comprising using a 5' cap having the sequence of $N1vpppN2pU$ and modifying at least one uridine in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine.

[0351] In some embodiments, the 5' cap has the sequence of $GvpppApU$, wherein A is modified or unmodified. In some embodiments, the A is 2'-O-methylated. In some embodiments, the 5' cap has the sequence of $m^7GvpppApU$. In some embodiments, the 5' cap has the sequence of $m^7GvpppApU$, wherein the A is modified or unmodified. In some embodiments, the A is 2'-O-methylated. In some embodiments, the 5' cap has the sequence of $m^7GvpppA_{mp}U$. In some embodiments, the 5' cap comprises a nucleic acid sequence set forth in any one of SEQ ID NOs: 21 or 34.

[0352] In some embodiments, $m^7GvpppA_{mp}U$ has the following formula,



[0353] In some embodiments, the method comprises modifying between about 1% and about 99% of the uridine bases in the RNA molecule. In some embodiments, the method comprises modifying about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% of the uridines in the RNA molecule. In some embodiments, the method comprises modifying at least about 50% of the uridines other than the first 5' uridine in the RNA molecule. In some embodiments, the

method comprises modifying at least about 90% of the uridines other than the first 5' uridine in the RNA molecule. In some embodiments, the method comprises modifying at least about 95% of the uridines other than the first 5' uridine in the RNA molecule. In some embodiments, the method comprises modifying at least about 99% of the uridines other than the first 5' uridine in the RNA molecule.

[0354] In some embodiments, the method comprises modifying all of the uridines in the RNA molecule except for the first 5' uridine.

[0355] In some embodiments, the method comprises modifying between about 1% and about 99% of the uridine bases in the RNA molecule to Ψ . In some embodiments, the method comprises modifying about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% of the uridines in the RNA molecule to Ψ . In some embodiments, the method comprises modifying at least about 50% of the uridines other than the first 5' uridine in the RNA molecule to Ψ . In some embodiments, the method comprises modifying at least about 90% of the uridines other than the first 5' uridine in the RNA molecule to Ψ . In some embodiments, the method comprises modifying at least about 95% of the uridines other than the first 5' uridine in the RNA molecule to Ψ . In some embodiments, the method comprises modifying at least about 99% of the uridines other than the first 5' uridine in the RNA molecule to Ψ . In some embodiments, the method comprises modifying all of the uridines other than the first 5' uridine in the RNA molecule to Ψ .

[0356] In some embodiments, the method comprises modifying between about 1% and about 99% of the uridine bases in the RNA molecule to m1 Ψ . In some embodiments, the method comprises modifying about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% of the uridines in the RNA molecule to m1 Ψ . In some embodiments, the method comprises modifying at least about 50% of the uridines other than the first 5' uridine in the RNA molecule to m1 Ψ . In some embodiments, the method comprises modifying at least about 90% of the uridines other than the first 5' uridine in the RNA molecule to m1 Ψ . In some embodiments, the method comprises modifying at least about 95% of the uridines other than the first 5' uridine in the RNA molecule to m1 Ψ . In some embodiments, the method comprises modifying at least about 99% of the uridines other than the first 5' uridine in the RNA molecule to m1 Ψ . In some embodiments, the method comprises modifying all of the uridines other than the first 5' uridine in the RNA molecule to m1 Ψ .

[0357] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of GpppApU and modifying at least one uridine in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0358] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of GpppApU and modifying all of the uridines in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0359] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of GpppApU and modifying all of the uridines in the RNA molecule to Ψ except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0360] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of GpppApU and modifying all of the uridines in the RNA molecule to m¹ Ψ except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0361] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GpppApU and modifying at least one uridine in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0362] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GpppApU and modifying all of the uridines in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0363] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GpppApU and modifying all of the uridines in the RNA molecule to Ψ except for the first 5' uridine to

produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0364] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GpppApU and modifying all of the uridines in the RNA molecule to m¹Ψ except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0365] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GpppA_mpU and modifying at least one uridine in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0366] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GpppA_mpU and modifying all of the uridines in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0367] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GpppA_mpU and modifying all of the uridines in the RNA molecule to Ψ except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0368] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GpppA_mpU and modifying all of the uridines in the RNA molecule to m¹Ψ except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0369] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of GvpppApU and modifying at least one uridine in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0370] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of GvpppApU and

modifying all of the uridines in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0371] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of GvpppApU and modifying all of the uridines in the RNA molecule to Ψ except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0372] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of GvpppApU and modifying all of the uridines in the RNA molecule to m¹ Ψ except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0373] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GvpppApU and modifying at least one uridine in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0374] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GvpppApU and modifying all of the uridines in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0375] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GvpppApU and modifying all of the uridines in the RNA molecule to Ψ except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0376] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GvpppApU and modifying all of the uridines in the RNA molecule to m¹ Ψ except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0377] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GvpppA_mpU and modifying at least one uridine in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0378] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GvpppA_mpU and modifying all of the uridines in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0379] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GvpppA_mpU and modifying all of the uridines in the RNA molecule to Ψ except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0380] In some embodiments, the method of lowering the innate immune stimulation of a RNA molecule comprises using a 5' cap having the sequence of m⁷GvpppA_mpU and modifying all of the uridines in the RNA molecule to m¹Ψ except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, and wherein U is an unmodified uridine.

[0381] In some embodiments, the production method of RNA molecule for lowering the innate immune stimulation of a RNA molecule as described above comprises the method of *in vitro* transcribing a DNA molecule to a modified RNA molecule disclosed herein.

[0382] In other aspects, provided is the use of the modified RNA molecule, the pharmaceutical composition, the method, the modified RNA molecule or the kit disclosed herein in lowering the innate immune stimulation of RNA delivery.

[0383] It is understood that any embodiment of the compounds provided herein, as set forth above, and any specific substituent and/or variable in the compound provided herein, as set forth above, may be independently combined with other embodiments and/or substituents and/or variables of the compounds to form embodiments not specifically set forth above. In addition, in the event that a list of substituents and/or variables is listed for any particular group or variable, it is understood that each individual substituent and/or variable may be deleted from the particular embodiment and/or claim and that the remaining

list of substituents and/or variables will be considered to be within the scope of embodiments provided herein.

[0384] It is understood that in the present description, combinations of substituents and/or variables of the depicted formulae are permissible only if such contributions result in stable compounds.

Beneficial Effects

[0385] In one aspect, the application of the modified RNA molecule, the pharmaceutical composition thereof, the modified RNA molecule produced by the method and the kit herein will achieve at least one of the following:

- (1) the capping efficiency will be enhanced;
- (2) the RIG-I activation will be reduced, i.e., significantly less immunostimulation, leading to lower innate immune stimulation when delivered to a cell.;
- (3) the low toxicity, high yield and high purity will be achieved;
- (4) the antigen-specific IgG titer will be enhanced.

EXAMPLES

[0386] The following examples are included for illustrative purposes only and are not intended to limit the scope of the present disclosure.

Example 1: *In vitro* transcriptional preparation of mRNA and detection of each index

[0387] The present disclosure is not intended to be limited in scope to the particular disclosed embodiments, which are provided, for example, to illustrate various aspects of the present disclosure. Various modifications to the compositions and methods described will become apparent from the description and teachings herein. Such variations may be practiced without departing from the true scope and spirit of the disclosure and are intended to fall within the scope of the present disclosure.

[0388] This example describes the *in vitro* transcription of modified RNA molecules and the analysis of mRNA purity, 5' capping efficiency, immunostimulation.

[0389] Plasmid linearization: DNA plasmid template containing DNA encoding eGFP, SARS-COV-2 Omicron BA.4/5 RBD, was linearized using restriction endonuclease. Purified by adding 1/10 volume of 3M sodium acetate (pH 5.5) and 2.5 times the volume of ethanol, washed twice with 70% ethanol and resuspended in nuclease-free water.

In vitro transcription:

[0390] The purified linear plasmid was used as a template for the *in vitro* transcription reaction using T7 RNA polymerase. *In vitro* transcription was performed by addition of m⁷GpppA_mpU or m⁷GpppA_mpG, respectively, to synthesize mRNA with a Cap1 "cap" structure, and the mRNAs were synthesized with mIΨ instead of uridine.

[0391] For the m⁷GpppAmpU, ATP/GTP/CTP/mIΨ each were at a concentration of 5 mM; m⁷GpppAmpU was at a concentration of 4 mM; 10X Reaction buffer [400 mM Tris-HCl (pH 7.9), 100 mM DTT, 20 mM spermidine, 0.02% Triton X-100, 270 mM Magnesium Acetate] was at a concentration of 1X; DNA template was at a concentration of 50 µg/mL; Murine RNase inhibitor was at a concentration of 1 unit/µL; Yeast Inorganic Pyrophosphatase was at a concentration of 0.002 unit/µL; T7 RNA Polymerase was at a concentration of 5 unit/µL. For the m⁷GpppAmpG, ATP/GTP/CTP/mIΨ each were at a concentration of 5 mM; m⁷GpppAmpG was at a concentration of 4 mM; 10X Reaction buffer [400 mM Tris-HCl (pH 7.9), 100 mM DTT, 20 mM spermidine, 0.02% Triton X-100, 270 mM Magnesium Acetate] was at a concentration of 1X; DNA template was at a concentration of 50 µg/mL; Murine RNase inhibitor was at a concentration of 1 unit/µL; Yeast Inorganic Pyrophosphatase was at a concentration of 0.002 unit/µL; T7 RNA Polymerase was at a concentration of 5 unit/µL.

[0392] The reactions took 2.5h, followed by DNase I digestion (DNase I with a final concentration of 2 units/µL was added to the IVT reaction and incubated at 37°C for 30 minutes). The mRNA was then purified using the Monarch® RNA Cleanup Kit (NEB, #T2050S) immediately, quantified by UV using NanoDrop One, analyzed for purity using Fragment Analyzer, and detected for cap rate using LC-MS.

[0393] Results: As shown in Table 5, the cap rates of the mRNAs synthesized based on m⁷GpppA_mpU were all higher than those based on m⁷GpppA_mpG, and the purity was basically the same.

Table 5:

No.	mRNA name	GOI	Cap analog	Uridine	mRNA purity (%)	Cap1 (%)
1	mRNA1 (SEQ ID NO:1)	eGFP	m ⁷ GpppA _m pG	mIΨ	93.1	94.57
2	mRNA2 (SEQ ID NO:2)		m ⁷ GpppA _m pU	mIΨ	91.7	98.04

3	mRNA3 (SEQ ID NO:3)	BA. 4/5	m ⁷ GpppA _{mp} G	m1Ψ	90.7	92.77
4	mRNA4 (SEQ ID NO:4)		m ⁷ GpppA _{mp} U	m1Ψ	97.3	97.38

RIG-I activation studies using mRNA synthesized by two cap analogues:

[0394] A reporter cell line overexpressing RIG-I was used to detect the difference in mRNA secretion of RIG-I. HEK Lucia RIG-I cells were purchased from Invivogen (catalog # hkl-hrigi) and cells were cultured at 37°C in 5% CO₂ in DMEM medium supplemented with 10% fetal bovine serum. Prior to transfection, cells were inoculated at a density of 20,000 cells per well (in 100 µl of medium) in 96-well plates until cell fusion reached 80% or more for transfection, and 250 ng mRNA was transfected using Lipofectamine 2000 (Invitrogen, catalog # 11668-019). 50 µL of cell supernatant was taken 24 h after transfection. Cell supernatant was collected into a new 96-well plate, and 10 µL of detection reagent QUANTI-Luc™ was added to each well. The OD at 560 nm was read by Molecular Devices plate reader and analyzed for data.

[0395] Results: As shown in Table 6, the RIG-I activation of the mRNA synthesized based on m⁷GpppA_{mp}U was lower than that of m⁷GpppA_{mp}G.

Table 6:

NO.	mRNA name	Sample	Uridine	Cap analog	RIG-I activation (RLU)
1	mRNA1 (SEQ ID NO:1)	cGFP	m1Ψ	m ⁷ GpppA _{mp} G	9572
2	mRNA2 (SEQ ID NO:2)		m1Ψ	m ⁷ GpppA _{mp} U	4947
3	mRNA3 (SEQ ID NO:3)	BA. 4/5	m1Ψ	m ⁷ GpppA _{mp} G	4044
4	mRNA4 (SEQ ID NO:4)		m1Ψ	m ⁷ GpppA _{mp} U	2948
5	/	Mock	N/A	N/A	284

Cytotoxicity study using mRNA synthesized by two cap analogues:

[0396] HEK-293T and A549 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum at 37°C, 5% CO₂, and inoculated with 100 µl of cell suspension per well (5000 cells/well) in 96-well plates. The plates are pre-incubated in a humidified incubator for 24 hr (37°C, 5% CO₂) and 10 µl of each mRNA is added to the wells. The plates are incubated in the incubator for 24 hr, 10 µl of CCK-8 solution (Catalog No.:

RM02823, abclonal) is added to each well of the plates and the plates are incubated in the incubator for 1-4 hr on a shaker. Mix gently. The absorbance at 450 nm was then measured using an enzyme marker.

[0397] RESULTS: As shown in Table 7, there was no significant toxicity difference between the two mRNAs for both cell lines.

Table 7:

NO.	mRNA name	Sample	Cap analog	HEK-293T Cell Viability	A549 Cell Viability
1	mRNA1 (SEQ ID NO:1)	eGFP	m ⁷ GpppA _m pG	96.2%	96.2%
2	mRNA2 (SEQ ID NO:2)		m ⁷ GpppA _m pU	95.5%	95.5%
3	/	PBS	N/A	100.0%	100.0%

Example 2: Example of a rabies virus-based mRNA vaccine

[0398] This example describes the *in vitro* transcription of a rabies virus-based mRNA vaccine.

[0399] According to the method of Example 1, mRNA encoding rabies virus antigen (named mRNA5 (SEQ ID NO: 5), mRNA6 (SEQ ID NO: 6), respectively) was prepared using m⁷GpppA_mpG and m⁷GpppA_mpU, respectively, and both of them were synthesized with m¹Ψ instead of uridine. Then, by dissolving a lipid mixture, including 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), ionizable lipids, PEG-lipids and cholesterol in ethanol and then mixed with mRNA solution in a T-mixer. The preparations are then concentrated to the desired concentration by tangential flow filtration (TFF) membranes and filtered through 0.22 μm filters to become finished products (named LNP1, LNP2, respectively), which are stored at 2-8°C until use. Using the finished products for experimental animals, BALB/c mice were injected with each preparation (0.1 μg/50 μL/mouse) on day 0 in a single spot intramuscularly in the right hind limb, 10 animals in each group (except for the PBS group with 5 animals), and whole blood was taken 13 days after administration to measure serum levels of RABV-specific IgG titer.

Results:

[0400] As shown in Table 8, LNP2 group had higher IgG titer level than the LNP1 group.

Table 8:

NO.	RABV-specific IgG titer		
	PBS	LNP1	LNP2
1	12	757	2957
2	7	2342	1056
3	14	181	2307
4	8	104	404
5	7	1505	932
6	/	2404	7541
7	/	2369	3541
8	/	195	5200
9	/	3719	1171
10	/	831	5001

Example 3: Example of a respiratory syncytial virus-based mRNA vaccine

[0401] This example describes the *in vitro* transcription of a respiratory syncytial virus-based mRNA vaccine.

[0402] According to the method of Example 1, mRNA encoding respiratory syncytial virus antigen (named mRNA7 (SEQ ID NO: 7), mRNA8 (SEQ ID NO: 8), respectively) was prepared using m⁷GpppA_mpG and m⁷GpppA_mpU, respectively, and both of them were synthesized with m¹Ψ instead of uridine. Then, by dissolving lipid mixtures, including 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), ionizable lipids, PEG-lipids and cholesterol in ethanol and then mixed with mRNA solution in a T-mixer. The preparations were then concentrated to the desired concentration by tangential flow filtration (TFF) membranes and filtered through 0.22 μm filters to become finished products (named LNP3, LNP4, respectively), which were stored at 2-8°C until use. Using the finished products for experimental animals, BALB/c mice were injected with each preparation (0.1 μg/50 μL/mouse) on day 0 in a single spot intramuscularly in the right hind limb, 5 animals for PBS, 6 animals for LNP3, and 10 animals for LNP4. Whole blood was taken 13 days after administration to measure serum levels of RSV-specific Ig G.

[0403] Results: As shown in Table 9, the LNP4 group had a higher IgG titer level than the LNP3 group.

Table 9:

NO.	RSV-specific IgG titer		
	PBS	LNP3	LNP4
1	45*	271.9201	1039.993
2	45*	435.411	1089.876
3	45*	463.083	1595.512
4	45*	1036.854	3036.083

5	45*	1036.007	3072.362
6	/	897.9293	3129.047
7	/	/	1414.118
8	/	/	2791.373
9	/	/	7564.132
10	/	/	8130.887

* Lower than detection limit

Example 4: Expression in BHK-21 cell-line

[0404] According to the method of Example 1, mRNAs encoding the eGFP protein were synthesized with $(G^{m7})ppp(A^{m2})G$ and $(G^{m7})ppp(A^{m2})U$ respectively, and both of them were synthesized with m $^1\Psi$ instead of uridine (i.e., mRNA1 (SEQ ID NO: 1), mRNA2 (SEQ ID NO: 2), respectively). BHK-21 cells were cultured in RPMI 1640 containing 10% fetal bovine serum (FBS; Fisher Scientific, Inc.), 100 U/ml penicillin, and 100 mg/ml streptomycin at 37°C with 5% CO₂. BHK-21 cells were seeded in 96-well plates at a density of 10,000 cells per well (in 100 μ L medium) 24h before transfection. 25 ng of mRNA were transfected with TransIT (Mirus bio, catalog # MIR 2250) and cells were collected and resuspended in PBS 24 h later. The value at 488/507 nm was read by Molecular Devices plate reader for detection, and the data were analyzed by Graphpad prism 9. The results were shown in FIG. 1, which indicated that the expression levels of eGFP in BHK-21 were similar in both mRNA and there was no significant difference between the groups.

Example 5: In vitro transcriptional preparation of mRNA with similar cap structure and detection of each mRNA

[0405] The purified linear plasmids encoding the RSV, RABV, VZV antigens and the eGFP protein were used as the templates for the in vitro transcription reaction using T7 RNA polymerase, respectively. The linear templates were prepared and purified as described in Example 1. In vitro transcription was performed by addition of $(G^{m7})ppp(A^{m2})U$, $(G^{m7,3'OMe})ppp(A^{m2})U$, $(G^{m7})ppp(A^{m2})m^1\Psi$ or $(G^{m7})vppp(A^{m2})U$, respectively, to synthesize mRNA with a Cap1 "cap" structure. Meanwhile, the mRNAs were synthesized with m $^1\Psi$ or uridine.

[0406] ATP/GTP/CTP/m $^1\Psi$ each were at a concentration of 12.5 mM; $(G^{m7})ppp(A^{m2})U$ or $(G^{m7,3'OMe})ppp(A^{m2})U$ or $(G^{m7})ppp(A^{m2})m^1\Psi$ or $(G^{m7})vppp(A^{m2})U$ were at a concentration of 12.5 mM; 10X Reaction buffer [400 mM Tris-HCl (pH 7.9), 100 mM DTT, 20 mM spermidine, 0.02% Triton X-100, 270 mM Magnesium Acetate] was at a concentration of 1X; DNA template was at a concentration of 50 μ g/mL; Murine RNase

inhibitor was at a concentration of 1 unit/ μ L; Yeast Inorganic Pyrophosphatase was at a concentration of 0.0005 unit/ μ L; T7 RNA Polymerase was at a concentration of 5 unit/ μ L.

[0407] The IVT reactions took 2.5 h, followed by DNase I digestion (DNase I with a final concentration of 2 units/ μ L was added to the IVT reaction and incubated at 37°C for 30 minutes). Table 11 provided the specific information about the mRNAs, such as structures of the different cap analogs. The cap analog products such as (G^{m7})ppp(A^{m2})U, (G^{m7,3'OMe})ppp(A^{m2})U, (G^{m7})ppp(A^{m2})m1 Ψ or (G^{m7})vppp(A^{m2})U are commercially available, and their methods of preparation are known in the art. For example, (G^{m7})vppp(A^{m2})U can be prepared according to CN116143855A or is available from Jiangsu Synthgene Biotechnology Co., Ltd.. The mRNA was then purified using the Monarch® RNA Cleanup Kit (NEB, #T2050S) immediately, quantified by UV using NanoDrop One, analyzed for purity using Fragment Analyzer, detected for cap rate using LC-MS and analyzed for RIG-I activation with HEK Lucia RIG-I cells purchased from Invivogen (catalog # hkl-hrigi).

[0408] Results analyzed from Table 10:

(1) Yield: The yield of the mRNAs synthesized based on (G^{m7})ppp(A^{m2})U was higher than that based on (G^{m7,3'OMe})ppp(A^{m2})U or (G^{m7})ppp(A^{m2})m1 Ψ , with good consistency between four groups of different mRNA samples. And the yield of mRNA based on (G^{m7})ppp(A^{m2})U was basically the same as that based on (G^{m7})vppp(A^{m2})U when encoding eGFP.

(2) Purity: The purity of the mRNAs synthesized based on (G^{m7})ppp(A^{m2})U was higher than that based on (G^{m7,3'OMe})ppp(A^{m2})U or (G^{m7})ppp(A^{m2})m1 Ψ , with good consistency between four groups of different mRNA samples. The purity of eGFP-coding mRNA synthesized based on (G^{m7})ppp(A^{m2})U was basically the same as that based on (G^{m7})vppp(A^{m2})U when using either uridine or m1 Ψ modification.

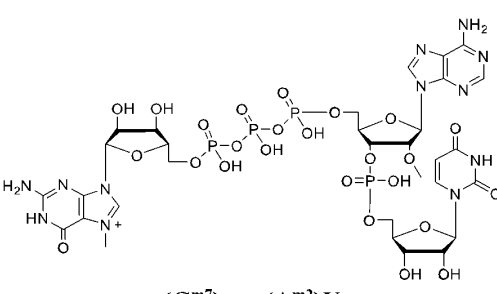
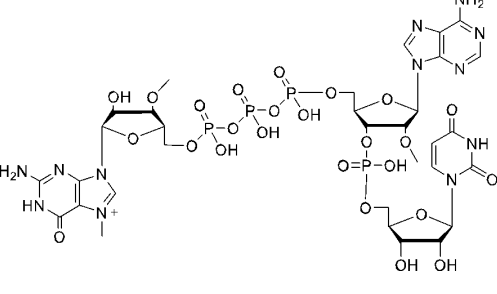
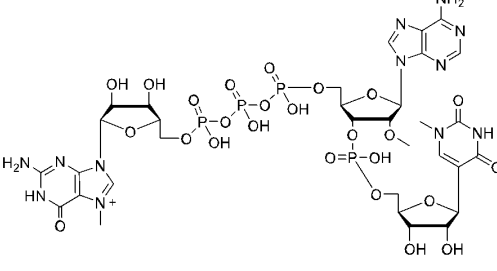
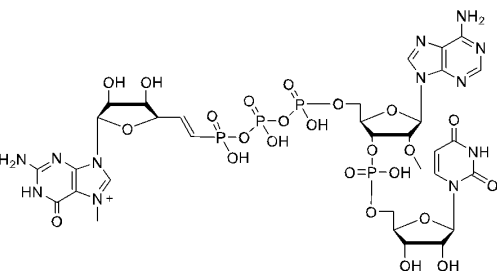
(3) Capping rate: The capping rates of the mRNAs synthesized based on all those cap analogs were equally high, up to 100%.

(4) RIG-I activation: When replacing uridine with m1 Ψ , the RIG-I activation of mRNAs based on (G^{m7})ppp(A^{m2})U was lower than that based on (G^{m7,3'OMe})ppp(A^{m2})U or (G^{m7})ppp(A^{m2})m1 Ψ , with good consistency between four groups of different samples. The RIG-I activation of eGFP-coding mRNA synthesized based on (G^{m7})ppp(A^{m2})U was basically the same as that based on (G^{m7})vppp(A^{m2})U when using either uridine or m1 Ψ modification.

Table 10:

NO.	mRNA name	Uridine	Sample	Cap analog	Yield ($\mu\text{g}/\text{Rxn}$)	Purity (%)	Capping rate (%)	RIG-1 activation (RLU)
1	mRNA9 (SEQ ID NO:9)	UTP	RSV	(G ^{m7})ppp(A ^{m2})U	219.86	92.6	99.67	15111
2	mRNA10 (SEQ ID NO:10)			(G ^{m7,3'OMe})ppp(A ^{m2})U	151.24	89	99.83	11570
3	mRNA11 (SEQ ID NO:11)			(G ^{m7})ppp(A ^{m2})m1 Ψ	207.57	87.7	100	8960
4	mRNA12 (SEQ ID NO:12)		VZV	(G ^{m7})ppp(A ^{m2})U	205.49	94	100	6319
5	mRNA13 (SEQ ID NO:13)			(G ^{m7,3'OMe})ppp(A ^{m2})U	175.37	92.4	99.97	5933
6	mRNA14 (SEQ ID NO:14)			(G ^{m7})ppp(A ^{m2})m1 Ψ	184.68	89.4	100	5423
7	mRNA15 (SEQ ID NO:15)		RABV	(G ^{m7})ppp(A ^{m2})U	225	92.1	99.21	10256
8	mRNA16 (SEQ ID NO:16)			(G ^{m7,3'OMe})ppp(A ^{m2})U	162.13	90.5	99.27	12012
9	mRNA17 (SEQ ID NO:17)			(G ^{m7})ppp(A ^{m2})m1 Ψ	211.35	85.7	99.28	7421
10	mRNA18 (SEQ ID NO:18)		eGFP	(G ^{m7})ppp(A ^{m2})U	192.5	92.2	99.97	11800
11	mRNA19 (SEQ ID NO:19)			(G ^{m7,3'OMe})ppp(A ^{m2})U	96.63	91	99.96	17178
12	mRNA20 (SEQ ID NO:20)			(G ^{m7})ppp(A ^{m2})m1 Ψ	159.09	90.2	99.98	11090
13	mRNA21 (SEQ ID NO:21)			(G ^{m7})vppp(A ^{m2})U	199.21	95.5	99.9	7070
14	mRNA22 (SEQ ID NO:22)	m1 Ψ	RSV	(G ^{m7})ppp(A ^{m2})U	197.58	90	99.72	1099
15	mRNA23 (SEQ ID NO:23)			(G ^{m7,3'OMe})ppp(A ^{m2})U	127.93	90.5	99.95	1140
16	mRNA24 (SEQ ID NO:24)			(G ^{m7})ppp(A ^{m2})m1 Ψ	161.12	90.1	99.64	/
17	mRNA25 (SEQ ID NO:25)		VZV	(G ^{m7})ppp(A ^{m2})U	190.85	93.7	100	592
18	mRNA26 (SEQ ID NO:26)			(G ^{m7,3'OMe})ppp(A ^{m2})U	149.2	89.3	100	903
19	mRNA27 (SEQ ID NO:27)			(G ^{m7})ppp(A ^{m2})m1 Ψ	181.43	84.6	100	618
20	mRNA28 (SEQ ID NO:28)		RABV	(G ^{m7})ppp(A ^{m2})U	223.06	93.6	99.95	1634
21	mRNA29 (SEQ ID NO:29)			(G ^{m7,3'OMe})ppp(A ^{m2})U	125.78	90.8	100	1952
22	mRNA30 (SEQ ID NO:30)			(G ^{m7})ppp(A ^{m2})m1 Ψ	158.91	89.8	99.98	1816
23	mRNA31 (SEQ ID NO:31)		eGFP	(G ^{m7})ppp(A ^{m2})U	179.56	92.6	99.91	2442
24	mRNA32 (SEQ ID NO:32)			(G ^{m7,3'OMe})ppp(A ^{m2})U	78.24	90.7	99.87	3790
25	mRNA33 (SEQ ID NO:33)			(G ^{m7})ppp(A ^{m2})m1 Ψ	109.01	87.7	100	/
26	mRNA34 (SEQ ID NO:34)			(G ^{m7})vppp(A ^{m2})U	190.87	95	100	1088

Table 11:

 (G^{m7})ppp(A^{m2})U	 (G^{m7,3'OMe})ppp(A^{m2})U
 (G^{m7})ppp(A^{m2})m1Ψ	 (G^{m7})vppp(A^{m2})U

Example 6: In vitro expression of mRNA synthesized by (G^{m7})ppp(A^{m2})U and (G^{m7})vppp(A^{m2})U

[0300] The mRNAs synthesized based on (G^{m7})ppp(A^{m2})U and (G^{m7})vppp(A^{m2})U and with either uridine or m1Ψ modification were prepared as described in Example 5. HEK-293T and A549 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum at 37°C, 5% CO₂, and inoculated with 100 μl of cell suspension per well (5000 cells/well) in 96-well plates. The plates were pre-incubated in a humidified incubator for 24 hr (37°C, 5% CO₂) and 100 ng of each mRNA was added to the wells. Cells were collected partially at 24 hr and 48 hr respectively, and were washed with PBS for three times. eGFP protein expression level was evaluated with Flow cytometer. As shown in FIG. 2-3, mRNAs synthesized based on (G^{m7})ppp(A^{m2})U and (G^{m7})vppp(A^{m2})U had basically the same expression level when using either uridine or m1Ψ modification.

In the following sequences, “Ψ” denotes “m1Ψ (1-methyl-pseudouridine)” unless otherwise indicated. The abbreviation is for the clarity of the sequences. The other abbreviations are as shown below,

“m⁷G” and “G^{m7}” = 7-methylguanosine (can also be abbreviated as “m7G”);
 “A_m” and “A^{m2}” = 2'-O-methyladenosine;
 “ppp” = triphosphate linkage;
 “vppp” = vinyl-modified triphosphate linkage;
 “G^{m7,3'OMe}” = 3'-O-methylated m7G.

[illegible][illegible]

m⁷GpppAmpGGAAAΨAAGGAΨCCΨΨGΨΨCΨΨΨΨΨGCGAGAAGCΨCAGAAΨAAACGCΨCAACΨΨΨGGCGCCGCCACCAΨGGACΨGGACCΨGGAΨACΨGΨΨCCΨGGΨGGCCGCCGCCACAGAGΨGCA CAGCCGGGΨCCAGCCAACCGAGAGCAΨCGΨGAGAΨΨCCCCAACAPAAACAAACCΨGΨGCCCCΨΨC

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GCGYAGCCCAACCAACYGAAACGACCYGYGCPYACCAACGYGYACGCCGACAGCPYCGYGA
CAGAGGCAACGAGGYGAGCCAGAPYCGCCCCAGGCCAGACCGGAAACAYCGCCGACYACAACYAC
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CGYGGYPCPYGAAFAAAGPYCAGAGYGGGCGGCACYAGYAAAAAAAAAAAAAAAAAAAAAAAAA
AAA

SEQ ID NO: 4 (MRNA 4)

m⁷GpppA_{mp}UGAAAFAAGGAFCCTPYGYPYCYPYPYGCAGAAGCPYAGAAFAAACGCPYCAACPY
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SEQ ID NO: 5 (MRNA 5)

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SEQ ID NO: 6 (MRNA 6)

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SEQ ID NO: 7 (MRNA 7)

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 GCACCYΨGYΨCYΨGCCYΨCAGCAAGGGGYACCYGYΨCCGCCYΨGCGGACAGGAYGGYAYΨACCYΨCAGY
 GAYΨCACAAYΨGAGCYΨGCCAAΨAYΨAAGGAGAAΨAAGYGCAAΨGGGACYΨACGCCAAGGYGA
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 CACCYΨGAGGGCGAGGYGAΨAAGAYCAAGAGYGCYΨCYΨGAGCACYΨAACAAGGCYΨGYΨCYΨG
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AA
AAAAAAAA

SEQ ID NO: 9 (MRNA 9)

(G^{m7})ppp(A^{m2})UGGGGAUCCGCUUGGUUUUGGUGAUUUUUGUCUGAUUUGCAGCCACCAUGGAGCUGC
UGAUCCUCAAGGCCAACGCCAUCACCACCAUUCUGACAGCCGUGACGUUCUGUUUUGCCAGCGG
GCAGAACAUACCGAGGAGUUCUACCAGAGCACCUGUUCUGCCGUGUCCAAGGGCUACCUGGGC
GCCUGCGCACCGGGUGGUACACCUCUGUAUCACCAUCGAGCUGUCUAAUCAAGGAGAUUA
AGUGCAACGGCACUGACGCCAAGGUGAAGCUGAUCAAGCAGGAGCUGGACAAGUACAAGAACG
CCGUGACUGACCUGCAGCUGCUCAUGCAGAGCACCCCGCCACCGGGAGUGGCAGCGCCAUCUG
CAGCGGCGUGGCCGUCUGCAAGGUGCUGCACCUCGAGGGCGAGGUGAAUAAGAUAAGAGUGC
UCUGCUGAGCACUAACAAGGCUGUGGUCAGUCUGUAAAUGGGGUGAGCGUGCUGACCUCAA
GGUGCUGGACCUGAAGAAUUACAUUGACAAGCAGCUGCUGCCUAUUCUGAAUAAGCAGAGCUG
CAGCAUCCCCAACAUUGAGACAGUUUACGAGUUUCAGCAGAAGAACAACAGGCUGCUUGAGAUC
ACACGGGAGUUCAGCGUGAACGCUGGGGUGAGCACUCCCGUGUCCACUUAUGUGAGACCAACU
CAGAGCUGCUGUCCUGAUCAACGACAUGCCUAUUACAACGACCAGAAGAUAUGAGCAA
CAACGUGCAGAUUCGUGAGACAGCAGUCUACAGCAUCAUGUGCAUCAUCAAGGAGGAGGUGCU
GGCGUACGUGGUGCAGCUGCCCCUGUAUGGGGUGAUCGACACCCUUGUUGGAAGCUGCACACU
UCUCCCGUGUGCACGACCAAUACGAAGGAGGGCAGCAACAUCUGUCUGACCCGGACAGAUCCGG
GCUGGUUUUGCGACAAUGCCGGCUCUGUCCUUCUCCCCAGGCUGAGACUUGCAAGGUGCA
GAGUAACCGGGUGUCUGCGACACCAUGAACUCCCGGACUCUGCCUCCGAGGUGAACCUGUGCA
ACGUGGACAUUUUCAACCCCAAGUACGAUUGCAAGAUCAUGACUAGCAAGACCGACGUGUCUUC
UAGCGUGAUUACCAGCCUGGGGGCGAUUGUGUCGUGCUACGGGAAGACGAAGUGUACAGCUUC
CAACAAGAACAGGGGCAUCAUCAAGACCUUCUCUAAUGGGUGCGACUACGUCAGCAAUAAAGGGC
GUCGACACCGUCAGCGUGGGGAACACCUUGUACUGCGUGAACAAGCAGGAGGGGCAGAGCCUGU
ACGUGAAGGGGGAGGCCAUUAUCAACUUCUACGACCCCCUGGUGUCCCCAGCGACGAGUUCGA
CGCCUCUAUCAGCCAGGUGAACGAGAAGAUCAAUCAGUCCCUGGCCUUAUUAGGAAGUCUGAU
GAGCUGCUGCACAACGUGAACGCCGGGAAGAGCACUACGAAUAUCAUGAUCACGACGAUCAUCA
UCGUGAUCAUUGUGAUCCUGCUCAGCCUGAUCGCCGUGGGGUGCUCUCCUGUACUGCAAGGCCAG
GAGACCCCCGUGACCCUGUCCAAGGAUCAGCUGAGCGGGAUCAACAAUAUCGUUUUCAGCAAC
UGAUAAAGAAUUCGUGCAAACUCGAUCACUAGCUCUGCGUGAUGUGGCAGAAGCGAAGGGAACC
AGGUUUUGCAAAAGUAACUGUGGUGAUGGAAAUGUGUUAGCCUCAGACACUACUGAGGUGGUUC
UUUCUAUCCUAGUACAGUCACUAGUAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AA

SEQ ID NO: 10 (MRNA 10)

(G^{m7,3'OMe})ppp(A^{m2})UGGGGAUCCGCUUGGUUUUGGUGAUUUUUGUCUGAUUUGCAGCCACCAUGGAGC
UGCUGAUCCUCAAGGCCAACGCCAUCACCACCAUUCUGACAGCCGUGACGUUCUGUUUUGCCAG
CGGGCAGAACAUACCGAGGAGUUCUACCAGAGCACCUGUUCUGCCGUGUCCAAGGGCUACCUG
GGCGCCUGCGCACCGGGUGGUACACCUCUGUAUCACCAUCGAGCUGUCUAAUCAAGGAGA
UUAAGUGCAACGGCACUGACGCCAAGGUGAAGCUGAUCAAGCAGGAGCUGGACAAGUACAAGA
ACGCCGUGACUGACCUGCAGCUGCUCAUGCAGAGCACCCCGCCACCGGGAGUGGCAGCGCCAU
CUGCAGCGGGGUGGCCGUCUGCAAGGUGCUGCACCUCGAGGGCGAGGUGAAUAAGAUAAGAG
UGCUCUGCUGAGCACUAACAAGGCUGUGGUCAGUCUGUCAAUUGGGGUGAGCGUGCUGACCUU
CAAGGUGCUGGACCUGAAGAAUUACAUUGACAAGCAGCUGCUGCCUAUUCUGAAUAAGCAGAG
CUGCAGCAUCCCCAACAUUGAGACAGUUUACGAGUUUCAGCAGAAGAACAACAGGCUGCUUGAG
AUCACACGGGAGUUCAGCGUGAACGCUGGGGUGACCACUCCCGUGUCCACUUAUGCUGACCA
ACUCAGAGCUGCUGUCCUGAUCAACGACAUGCCUAUUACAACGACCAGAAGAAGUUGAUGAG
CAACAACGUGCAGAUUCGUGAGACAGCAGUCUUAACAGCAUCAUGUGCAUCAUCAAGGAGGAGGU
GCUGGCGUACGUGGUGCAGCUGCCCCUGUAUGGGGUGAUCGACACCCCUUGUUGGAAGCUGCAC
ACUUCUCCCCUGUGCACGACCAAUACGAAGGAGGGCAGCAACAUCUGUCUGACCCGGACAGAU
GGGGCUGGUUUUGCGACAAUGCCGGCUCGUGUCCUUCUCCCCAGGCUGAGACUUGCAAGGU
GCAGAGUAACCGGGUGUCUGCGACACCAUGAACUCCCGGACUCUGCCUCCGAGGUGAACCUGU

GCAACGUGGACAUUUUCAACCCCAAGUACGAUUGCAAGAUCAUGACUAGCAAGACCGACGUGUC
 UUCUAGCGUGAUUACCAGCCUGGGGGCGAUUGUGUCGUGCUACGGGAAGACGAAGUGUACAGC
 UUCCAACAAGAACAGGGGCAUCAUCAAGACCUUCUCUAAUGGGUGCGACUACGUCAGCAAUAAAG
 GCGUCGACACCGUCAGCGUGGGGAACACCUUGUACUGCGUGAACAAGCAGGAGGGGCAGAGCC
 UGUACGUGAAGGGGGAGCCCAUUUCAACUUCUACGACCCCUUGGUGUCCCCAGCGACGAGUU
 CGACGCCUCUAUCAGCCAGGUGAACGAGAAGAUCAAUCAGUCCCUUGGCCUUCAUUAGGAAGUCU
 GAUGAGCUGCUGCACAACGUGAACGCCGGGAAGAGCACUACGAAUAUCAUGAUCACGACGAUCA
 UCAUCGUGAUCAUUGUGAUCCUGCUCAGCCUGAUCGCCGUGGGGCGUGCUCCUGUACUGCAAGGC
 CAGGAGCACCCCGUGACCCUGUCCAAGGAUCAGCUGAGCGGGAUCAACAAUAUCGCUUUCAGC
AACUGAUAAAGAAUUCGUGCAAACUCGAUCACUAGCUCUGCGUGAUGUGGCAGAAGCGAAGGGA
ACCAGGUUUGCAAAAGUAACUGUGGUGAUGGAAAUGUGUUAGCCUCAGACACUACUGAGGUGG
UUCUUUCUAUCCUAGUACAGUCACUAGUAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AA

SEQ ID NO: 11 (MRNA 11)

(G^{m7})ppp(A^{m2})ΨGGGGAUCCCGUGGUUUUGGUGAUUUUUGUCUGAUUGCAGCCACCAUGGAGCUG
 CUGAUCCUCAAGGCCAACGCCAUCAACCAUUCUGACAGCCGUGACGUUCUGUUUUGCCAGCG
 GGCAGAACAUCAACCGAGGAGUUCUACCAGAGCACCUGUUCUGCCGUGUCCAAGGGCUACCUGGG
 CGCCUUGCGCACCGGGUGGUACACCUCCGUGAUCACCAUCGAGCUGUCUAAACAUCAAGGAGAUU
 AAGUGCAACGGCACUGACGCCAAGGUGAAGCUGAUCAAGCAGGAGCUGGACAAGUACAAGAAC
 GCCGUGACUGACCUGCAGCUGCUCUAGCAGAGCACCCCGCCACCGGGAGUGGCAGCGCCAUCU
 GCAGCGGCGUGGCCGUGCAAGGUGCUGCACCUCGAGGGCGAGGUGAAUAAGAUCAAGAGUG
 CUCUGCUGAGCACUAACAAGGUGUGGUCAGUCUGUCAAUUGGGGUGAGCGUGCUGACCUUCA
 AGGUGCUGGACCUGAAGAAUUAUUGACAAGCAGCUGCUGCCUAAUUCUGAAUAAGCAGAGCU
 GCAGCAUCCCCAACAUUGAGACAGUUUUCGAGUUUUCAGCAGAAGAACAACAGGCUGCUUGAGA
 UCACACGGGAGUUCAGCGUGAACGCUGGGGUGACCACUCCCGUGUCCACUUACAUGCUGACCAA
 CUCAGAGCUGCUGUCCUGAUCAACGACAUGCCUAAUUAACAACGACCAGAAGAAGUUGAUGAGC
 AACAACGUGCAGAUUCGUGAGACAGCAGUCUUAACAGCAUCAUGUGCAUCAUAAGGAGGAGGUG
 CUGGCGUACGUGGUGCAGCUGCCCCUGUAUGGGGUGAUCGACACCCCUUGUUGGAAGCUGCACA
 CUUCUCCCCUGUGCAGACCAAUACGAAGGAGGGCAGCAACAUCUGUCUGACCCGGACAGAUUCG
 GGGCUGGUAAUUGCGACAAUGCCGGCUCCGUGUCCUUCUCCCCCAGGCUGAGACUUGCAAGGUG
 CAGAGUAACCGGGUGUCUGCGACACCAUGAACUCCCGGACUCUGCCCUCCGAGGUGAACCUGUG
 CAACGUGGACAUUUUCAACCCCAAGUACGAUUGCAAGAUAUGACUAGCAAGACCGACGUGUCU
 UCUAGCGUGAUUACCAGCCUGGGGGCGAUUGUGUCGUGCUACGGGAAGACGAAGUGUACAGCU
 UCCAACAAGAACAGGGGCAUCAUCAAGACCUUCUCUAAUGGGUGCGACUACGUCAGCAAUAAGG
 GCGUCGACACCGUCAGCGUGGGGAACACCUUGUACUGCGUGAACAAGCAGGAGGGGCAGAGCCU
 GUACGUGAAGGGGGAGCCCAUUUCAACUUCUACGACCCCUUGGUGUCCCCAGCGACGAGUUC
 GACGCCUCUAUCAGCCAGGUGAACGAGAAGAUCAAUCAGUCCCUUGGCCUUAUUAAGGAAGUCUG
 AUGAGCUGCUGCACAACGUGAACGCCGGGAAGAGCACUACGAAUAUCAUGAUCACGACGAUCAU
 CAUCGUGAUCAUUGUGAUCCUGCUCAGCCUGAUCGCCGUGGGGCGUGCUCCUGUACUGCAAGGCC
 AGGAGCACCCCGUGACCCUGUCCAAGGAUCAGCUGAGCGGGAUCAACAAUAUCGCUUUCAGCA
 ACUGAUAAAGAAUUCGUGCAAACUCGAUCACUAGCUCUGCGUGAUGUGGCAGAAGCGAAGGGAA
CCAGGUUUGCAAAAGUAACUGUGGUGAUGGAAAUGUGUUAGCCUCAGACACUACUGAGGUGGU
UCUUUCUAUCCUAGUACAGUCACUAGUAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AA

SEQ ID NO: 12 (MRNA 12)

(G^{m7})ppp(A^{m2})UGGGGAUCCCGUAACUGGGUUAGGUGCCCGUGUUGCUGCUCGUGUUGAAUCUAGA
ACCGUAGCCAGACGCCACCAUGGACUGGACCUGGAUUCUGUCCUGGUGGCCGCCGCCACCAGG
 GUGCACAGCUCUGUCCUGAGGUACGACGACUUCACAUCAUGAGGACAAGCUGGACACCAACA
 GCGUGUACGAGCCUACUACCACAGCGACCACGCCGAGUCCUCCUGGGUGAAUAGGGGCGAGUC
 UUCCAGGAAGGCCUAUGACCACAAUAGCCCCUACAUCUGGCCCGGAACGACUACGACGGCUUC

CUGGAGAACGCCACGAGCACCACGGGGUGUACAACCAGGGCCGCGGGAUCGAUUCGGGGAGA
 GGCUGAUGCAGCCUACCCAGAUUCCGCCAGGAGGACCUGGGCGACGAUACCGGCAUUCACGU
 GAUCCCCACCCUGAAUGGGGACGAUCGGCACAAGAUCGUGAAUGUGGAUCAGCGCCAGUACGGC
 GAUGUGUUAAGGGGCGACCUGAAUCCCAAGCCCCAGGGCCAGCGGCUGAUCGAGGUGAGCGUGG
 AGGAGAAUCACCCUUUUACCCUGCGGGCCCCCAUUCAGAGGAUCUACGGGGUGCGGUACACCGA
 AACCUGGAGCUUCCUGCCCAGCCUGACCUGUACCGGGCGACGCCGCCCCGCCAUCCAGCACAUUCU
 GCCUGAAGCAUACCACCUGCUUCCAGGACGUGGUGGUGGACGUGGACUGCGCCGAGAAUACCAA
 GGAGGAUCAGCUGGCAGAGAUACAGCUACCGGUUUCAGGGGAAGAAGGAGGCGGACCAGCCUUG
 GAUCGUGGUGAACACCAGCACCUGUUCGACGAGCUGGAGCUGGACCCUCCGAGAUUCGAGCCC
 GCGUGUCUGAAGGUGCUGAGGACCGAGAAGCAGUACCUGGGGGUGUACAUCUGGAACAUCCGG
 GGGAGCGAUGGCACCAGCACAUACGCCACCUUCCUGGUGACCUGGAAGGGGGACGAGAAGACUC
 GGAACCCACCCUGCUGUGACCCACAGCCCAGGGGGCGCUGAGUCCACAUGUGGAACUACCA
 UAGCCACGUGUUCAGCGUGGGGGACACCUUCUCCUGGCUAUGCACCUGCAGUACAAGAUCAC
 GAGGCCCCUUUGAUUCUGCUGCUGGAGUGGCUGUACGUGCCCAUCGACCCACCCUGUCAGCCUA
 UGAGGCUGUACAGCACCUGCCUGUACCACCCCAACGCCCCACAGUGCCUGUCCACAUGAACAG
 CGGGUGCACCUCACCAGCCCCCACCUGGCCCAGAGGGUGGCCUCCACCUGUACCAGAAUUGC
 GAGCACGCCGACAAUACACCGCCUACUGUCUGGGGAUCAGCCACAUGGAGCCCAGCUUCGGGC
 UGAUCCUGCAGCAGCGGGGGACCACCCUGAAGUUCGUGGACACCCUGAGAGCCUGAGCGGGCU
 GUACGUGUUCGUGGUGUACUUUAACGGGCACGUGGAGGCCUGGCCUACACCGUGGUGUCCACC
 GUGGACCACUUCGUGAACGCCAUCGAGGAGAGGGGCUUCCUCCUACCGCCGGGCAGCCCCUG
 CCACCACCAAGCCCAAGGAGAUACCCCCGUGAACCCGGCACCAGCCCCUGAUCCGGUACGCC
 GCCUGGACAGGCGGGCUGGCUGCCGUGGUGCUGCUGGCCUGGUGAUCUCCUGAUCUGCACC
 CCAAGCGGAUGCGGGUGAAGGCCGCCAGGGUGGACAAGAGCCCUACAUAUCAGAGCAUAGUACCG
 GCGUGGCUGCCUGAUAAAGAAUUCUAGGCUGGAGCCUCGGUGGCCAUGCUUCUCCCCUUGG
 GCCUCCCCCAGCCCCUCCUCCUCCUGCACCUGUACCCCGUGGUCUUGAAUAAAGUCUGA
 GUGGGCGGCACUAGUAA
 AAA

SEQ ID NO: 13 (MRNA 13)

(G^{m7,3'OMe})ppp(A^{m2})UGGGGAUCCUGAACUGGGUUAAGGUGCCGCGUGUUGCUGCUCGUGUUGAAUCU
 AGAACCGUAGCCAGACGCCACCAUGGACUGGACCUGGAUUCUGUUCUGGUGGCCGCCGCCACC
 AGGGUGCACAGCUCUGUCCUGAGGUACGACGACUCCACAUCGAUGAGGACAAGCUGGACACCA
 ACAGCGUGUACGAGCCCUACUACCACAGCGACCACGCCGAGUCCUCCUGGGUGAAUAGGGGCGA
 GUCUCCAGGAAGGCCUAGACCACAUAAGCCCUACAUCUGGCCCCGGAACGACUACGACGGC
 UUCUGGAGAACGCCACGAGCACCACGGGGUGUACAACCAGGGCCGCGGAUCGAUUCGGGG
 AGAGGCUGAUGCAGCCUACCCAGAUUCCGCCAGGAGGACCUGGGCGACGAUACCGGCAUUCA
 CGUGAUCCCCACCCUGAAUGGGGACGAUCGGCACAAGAUCGUGAAUGUGGAUCAGCGCCAGUAC
 GGCGAUGUGUUAAGGGCGACCUGAAUCCCAAGCCCCAGGGCCAGCGGCUGAUCGAGGUGAGCG
 UGGAGGAGAAUACCCUUUUACCCUGCGGGCCCCCAUUCAGAGGAUCUACGGGGUGCGGUACAC
 CGAAACCUGGAGCUUCCUGCCCAGCCUGACCUGUACCGGCGACGCCGCCCCGCCAUCCAGCACA
 UCUGCCUGAAGCAUACCACCUGCUUCCAGGACGUGGUGGUGGACGUGGACUGCGCCGAGAAUAC
 CAAGGAGGAUCAGCUGGCAGAGAUACGCUACCGGUUUCAGGGGAAGAAGGAGGCCGACCAGCC
 UUGGAUCGUGGUGAACACCAGCACCUGUUCGACGAGCUGGAGCUGGACCUCUCCGAGAUUCGAG
 CCCGGCGUGCUGAAGGUGCUGAGGACCGAGAAGCAGUACCUGGGGGUGUACAUCUGGAACAUG
 CGGGGGAGCGAUGGCACCAGCACAUACGCCACCUUCCUGGUGACCUGGAAGGGGGACGAGAAGA
 CUCGGAACCCACCCUGCUGUGACCCACAGCCCAGGGGCGCUGAGUCCACAUGUGGAACUA
 CCAUAGCCACGUGUUCAGCGUGGGGGACACCUUCUCCUGGCUAUGCACCUGCAGUACAAGAUC
 CACGAGGCCCCCUUGAUCUGCUGCUGGAGUGGCUGUACGUGCCCAUCGACCCACCUGUCAGC
 CUAUGAGGCUGUACAGCACCUGCCUGUACCACCCCAACGCCCCACAGUGCCUGUCCCAUGAA
 CAGCGGGUGCACCUCACACAGCCCCCACCUGGCCAGAGGGUGGCCUCCACCUGUACCAGAAU
 UGCGAGCACGCCGACAAUACACCGCCUACUGUCUGGGGAUCAGCCACAUGGAGCCAGCUUCG
 GGCUGAUCUGCAGCAGCGGGGGACACCCUGAAGUUCGUGGACACCCUGAGAGCCUGAGCGG
 GCUGUACGUGUUCGUGGUGUACUUUAACGGGCACGUGGAGGCCGUGGCCUACACCUGGUGUCC

ACCGUGGACCACUUCGUGAACGCCAUCGAGGAGAGGGGCUUCCCUCCUACCGCCGGGCAGCCCC
CUGCCACCACCAAGCCCAAGGAGAUCACCCCGUGAACCCCGGCACCAGCCCCUGAUCCGGUAC
GCCGCCUGGACAGGCGGGCUGGCUGCCGUGGUGCUGCUGGUGGUGAUCUUCUGAUCUGCA
CCGCCAAGCGGAUGCGGGUGAAGGCCGCCAGGGUGGACAAGAGCCCCUACAAUCAGAGCAUGUA
CGGGGCGUGGGCUGCCUGAUAAAGAAUUCUAGGCUGGAGCCUCGGUGGGCAUGCUUCUUGCCCCU
UGGGCCUCCCCCAGCCCCUCCUCCCUUCCUGCACCCGUACCCCGUGGUCUUUGAAUAAAGUC
UGAGUGGGCGGCACUAGUAAA
AA

SEQ ID NO: 14 (MRNA 14)

(G^{m7})ppp(A^{m2})ΨGGGGGAUCCUGAACUGGGUUAGGUGCCGCUGUUGCUGCUCGUGUUGAAUCUAG
AACCUGAGCCAGACGCCACCAUGGACUGGACCUGGAUUCUGUUCUGGUGGCCGCCGCCACCAG
GGUGCACAGCUCUGUCCUGAGGUACGACGACUCCACAUCGAUGAGGACAAGCUGGACACCAAC
AGCGUGUACGAGCCCUACUACCACAGCGACCACGCCGAGUCCUCCUGGGUGAAUAGGGGCGAGU
CUUCCAGGAAGGCCUAGUACCAAAUAGCCCUACAUUCUGGCCCCGGAACGACUACGACGGCUU
CCUGGAGAACGCCACGAGCACCACGGGGUGUACAACCAGGGCCGCGGGAUCGAUUCGGGGAG
AGGCUGAUGCAGCCUACCCAGAUUCCGCCCAGGAGGACCUGGGCGACGAUACCGGCAUUCACG
UGAUCCCCACCCUGAAUUGGGGACGAUCGGCACAAGAUCGUGAAUGUGGAUCAGCGCCAGUACGG
CGAUGUGUUCAAGGGCGACCUGAAUCCCAAGCCCCAGGGCCAGCGGCUGAUCGAGGUGAGCGUG
GAGGAGAAUACCCUUUUACCCUGCGGGCCCCCAUUCAGAGGAUCUACGGGGUGCGGUACACCG
AAACCUGGAGCUUCCUGCCCAGCCUGACCUGUACCGGGCAGCCGCCCCCCGCCAUCCAGCACAU
UGCCUGAAGCAUACCACCUUCUCCAGGACGUGGUGGUGGACGUGGACUGCGCCGAGAAUACCA
AGGAGGAUCAGCUGGCAGAGAUCAGCUACCGGUUUACAGGGGAAGAAGGAGGCCGACCAGCCUU
GGAUCGUGGUGAACACCAGCACCCUGUUCGACGAGCUGGAGCUGGACCCUCCCGAGAUCGAGCC
CGGCGUGCUGAAGGUGCUGAGGACCGAGAAGCAGUACCUUGGGGGUGUACAUCUGGAACAUGCG
GGGGAGCGAUGGCACCAGCACAUACGCCACCUUCCUGGUGACCUGGAAGGGGGACGAGAAGACU
CGGAACCCACCCCUUCUGUGACCCACAGCCCAGGGGCGCUGAGUCCACAUGUGGAACUACC
AUAGCCACGUGUUCAGCGUGGGGGACACCUUCUCCUGGCUAUGCACCUGCAGUACAAGAUA
CGAGGCCCCCUUGAUUCUGCUGCUGGAGUGGCUUACGUGCCCAUCGACCCACCCUGUCAGCCU
AUGAGGCUUACAGCACCUGCCUGUACACCCCAACGCCCCACAGUGCCUGUCCACAUGAACA
GCGGGUGCACCUUACACAGCCCCACCUGGCCCAGAGGGUGGCCUCCACCGUGUACCAGAAUUG
CGAGCACGCCGACAAUACACCGCCUACUGUCUGGGGAUCAGCCACAUGGAGCCCAGCUUCGGG
CUGAUCCUGCACGACGGGGGACCACCCUGAAGUUCUGGACACCCUGAGAGCCUGAGCGGGC
UGUACGUGUUCGUGGUGUACUUUAACGGGCACGUGGAGGCCGUGGCCUACACCGUGGUGUCCAC
CGUGGACCACUUCGUGAACGCCAUCGAGGAGAGGGGCUUCCCUCCUACCGCCGGGCAGCCCCU
GCCACCACCAAGCCCAAGGAGAUCACCCCGUGAACCCCGGCACCAGCCCCUGAUCCGGUACGC
CGCCUGGACAGGCGGGCUGGCUGCCGUGGUGCUGCUGGCCUGGUGAUCUUCUGAUCUGCACC
GCCAAGCGGAUGCGGGUGAAGGCCGCCAGGGUGGACAAGAGCCCCUACAUCAGAGCAUGUACG
GGGUGGGCUGCCUGAUAAAGAAUUCUAGGCUGGAGCCUCGGUGGGCAUGCUUCUUGCCCCUUG
GGCCUCCCCCAGCCCCUCCUCCCUUCCUGCACCCGUACCCCGUGGUCUUUGAAUAAAGUCUG
AGUGGGCGGCACUAGUAAA
AA

SEQ ID NO: 15 (MRNA 15)

(G^{m7})ppp(A^{m2})UGGGGAUCCAAGCUUAGCUUGCUUGUUCUUUUUGCAGAAGCUCAGAAUAAACGCU
CAACUUUGGCGCCGCCACCAUGGUGCCCCAAGCCUGCUGUUCGUGCCCCUGCUGGGCUUCAGC
CUGUGCUUCGGCAAGUCCCCAUCUACACCAUCCCCGACGAGCUGGGCCCCUGGAGCCCCAUCG
ACAUCCACCACCUGAGCUGCCCCAACAACCUUGGUGGUCGAGGAUGAAGGCUGCACCAACCUGAG
CGAGUUCAGCUACAUGGAGCUGAAGGUGGGCUACAUCAGCGCCAUAAGGUGAACGGCUUCACC
UGCACCGGCGUGGUGACCGAGGCCGAAACCUACACCAACUUCGUGGGCUACGUGACCACAACCU
UCAAGAGAAAGCACUUCAGACCCACCCCGACGCCUGCAGAGCCGCCUACAACUGGAAGAUGGC
CGGCGACCCUAGAUACGAGGAGAGCCUGCAUAAACCUUACCCCGACUACCACUGGCUGAGAACC

GUGAGAACCACCAAGGAGAGCCUGAUCAUUAUCAGCCCUAGCGUGACCGACCUGGACCCCUACG
ACAAAUCCUGCACAGCAGAGUGUUCGGCGGCAAGUGCAGCGGCAUCACCGUGAGCAGCAC
CUACUGCAGCACCAACCACGACUACACCAUCUGGAUGCCCGAGAACCCUAGACCUAGAACCCCC
UGCGACAUCUUCACCAACAGCAGAGGCAAGAGAGCUAGCAAGGGCAACAAGACCUAGCGGCUUCG
UGGACGAGAGAGGCCUGUACAAGAGCCUGAAGGGCGCCUGCAGACUGAAGCUGUGCGGCGUGC
UGGGCCUGAGACUGAUGGACGGCACCUGGGUGGCCAUGCAGACAAGCGACGAAACCAAGUGGU
GCCCCCGAUCAGCUGGUGAACCUGCACGACUUCAGAAGCGACGAGAUACGAGCACCUGGUGGU
GGAGGAGCUGGUGAAGAAGAGAGAGGAGUGCCUGGACGCCUGGAGAGCAUCAUGACCACCAA
GAGCGUGUCCUUCAGAAGACUGAGCCACCUGAGAAAGCUGGUGCCCGGCUUCGGCAAGGCCUAC
ACCAUCUUAACAAGACCCUGAUGGAGGCCGACGCCACUACAAAAGCGUGAGAACCUGGAACG
AGAUCAUCCUAGCAAGGGCUGCCUGAAGGUGGGCGGCAGAUGCCACCCCAUGUGAACGGCGU
GUUCUUAACGGCAUCAUCCUGGGCCCCGACGGCCACGUGCUGAUCCCCGAGAUAGCAGAGCAGC
CUGCUGCAGCAGCACAUUGGAGCUGCUGAAGAGCAGCGUGAUCCCCCUGAUGCACCCCUUGGCCG
ACCCUAGCACCGUGUUAAGGAGGGCGACGAGGCCGAGGACUUCGUGGAGGUGCACCUGCCCGA
CGUGUACAAGCAGAUACGCGGCGUGGACCUGGGCCUGCCCAACUGGGGCAAGUACGUGCUGAUG
ACCGCCGGCGCCAUGAUCGGCCUGGUGCUGAUUCUUCAGCCUGAUGACCUGGUGCAGAAGAGCCA
ACAGACCCGAGAGCAAGCAGCGGAGCUUCGGCGGCACCGGCAGAAACGUGAGCGUGACAAGCCA
AAGCGGCAAGGUGAUCCCUAGCUGGGAGAGCUACAAGAGCGGCGGGCAGACAAGACUGUGAU
AGAAUUCUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCAGCCCCUC
CUCCCCUCCUGCACCCGUACCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGCACUAGUAA
AA
AAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 16 (MRNA 16)

(G^{m7,3'OMe})ppp(A^{m2})UGGGGAUCCAAGCUUAGCUUGCUUGUUCUUUUUGCAGAAGCUCAGAAUAAAC
GCUCAACUUUGGCGCCGCCACCAUGGUGCCCCAAGCCCUGCUGUUCGUGCCCCUUCUGGGCUUC
AGCCUGUGCUUCGGCAAGUUCUACACCAUCCCCGACGAGCUGGGCCCCUGGAGCCCCA
UCGACAUCCACCACCUGAGCUGCCCCAACACCUUGGUGGUCGAGGAUGAAGGCGCACCACCU
GAGCGAGUUCAGCUACAUGGAGCUGAAGGUGGGCUACAUCAGCGCCAUAAGGUGAACGGCUU
CACCUGCACCGGCGUGGUGACCGAGGCCGAAACCUACACCAACUUCGUGGGCUACGUGACCACA
ACCUUCAAGAGAAAGCACUUCAGACCCACCCCGACGCCUGCAGAGCCGCCUACAACUGGAAGA
UGGCCGGCGACCCUAGAUACGAGGAGAGCCUGCAUAACCCUUAACCCGACUACCACUGGCUGAG
AACCGUGAGAACCACCAAGGAGAGCCUGAUCAUUAUCAGCCCUAGCGUGACCGACCUGGACCCC
UACGACAAAUCCUGCACAGCAGAGUGUUCGGCGGCAAGUGCAGCGGCAUCACCGUGAGCA
GCACCUACUGCAGCACCAACCACGACUACACCAUCUGGAUGCCCAGAAACCUAGACCUAGAAC
CCCCUGCGACAUUCUACCAACAGCAGAGGCAAGAGAGCUAGCAAGGGCAACAAGACCUAGCGGC
UUCGUGGACGAGAGAGGCCUGUACAAGAGCCUGAAGGGCGCCUGCAGACUGAAGCUGUGCGGC
GUGCUGGGCCUGAGACUGAUGGACGGCACCUUGGGUGGCCAUGCAGACAAGCGACGAAACCAAGU
GGUGCCCCCGAUCAGCUGGUGAACCUAGCAGCUUCAGAAGCGACGAGAUACGAGCACCUGGU
GGUGGAGGAGCUGGUGAAGAAGAGAGAGGAGUGCCUGGACGCCUGGAGAGCAUCAUGACCAC
CAAGAGCGUGUCCUUCAGAAGACUGAGCCACCUGAGAAAGCUGGUGCCCCGGCUUCGGCAAGGCC
UACACCAUCUUAACAAGACCCUGAUGGAGGCCGACGCCACUACAAAAGCGUGAGAACCUGGA
ACGAGAUCAUCCCUAGCAAGGGCUGCCUGAAGGUGGGCGGCAGAUGCCACCCCAUGUGAACGG
CGUGUUCUUAACGGCAUCAUCCUGGGCCCCGACGGCCACGUGCUGAUCCCCGAGAUAGCAGAGC
AGCCUGCUGCAGCAGCAUUGGAGCUGCUGAAGAGCAGCGUGAUCCCCCUGAUGCACCCCUUGG
CCGACCCUAGCACCGUGUUAAGGAGGGCGACGAGGCCGAGGACUUCGUGGAGGUGCACCUGCC
CGACGUGUACAAGCAGAUACGCGGCGUGGACCUGGGCCUGCCCAACUGGGGCAAGUACGUGCUG
AUGACCGCCGGCGCCAUGAUCGGCCUGGUGCUGAUUCUUCAGCCUGAUGACCUGGUGCAGAAGAG
CCAACAGACCCGAGAGCAAGCAGCGGAGCUUCGGCGGCACCGGCAGAAACGUGAGCGUGACAAG
CCAAAGCGGCAAGGUGAUCCCUAGCUGGGAGAGCUACAAGAGCGGCGGGCAGACAAGACUGUG
AUAAGAAUUCUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCAGCCC
CUCUCCCCUCCUGCACCCGUACCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGCACUAG

UAAA
 AAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 17 (MRNA 17)

(G^{m7})ppp(A^{m2})ΨGGGGAUCCAAGCUUAGCUUGCUUGUUCUUUUUGCAGAAGCUCAGAAUAAACGC
UCAACUUUGGCGCCGCCACCAUGGUGCCCCAAGCCUCUGUUCGUGCCCCUGUGGGCUUCAG
CCUGUGCUUCGGCAAGUCCCCAUCUACACCAUCCCCGACGAGCUGGGCCCCUGGAGCCCCAUC
GACAUCCACCACCUGAGCUGCCCCAACCAACCUGGUGGUCGAGGAUGAAGGCUGCACCAACCUGA
GCGAGUUCAGCUACAUGGAGCUGAAGGUGGGCUACAUCAGCGCCAUCAAGGUGAACGGCUUCAC
CUGCACCGGCGUGGUGACCGAGGCCGAAACCUACACCAACUUCGUGGGCUACGUGACCACAACC
UUCAAGAGAAAGCACUUCAGACCCACCCCGACGCCUGCAGAGCCGCCUACAACUGGAAGAUGG
CCGGCGACCCUAGAUACGAGGAGAGCCUGCAUAACCCUUAACCCGACUACCACUGGCUGAGAAC
CGUGAGAACCACCAAGGAGAGCCUGAUCAUUAUCAGCCUAGCGUGACCGACCUGGACCCCUAC
GACAAUCCUGCAGCAGCAGAGUGUUCGGCGGCAAGUGCAGCGGCAUACCCGUGAGCAGCA
CCUACUGCAGCACCAACCACGACUACACCAUCUGGAUGCCGAGAACCCUAGACCUAGAACCCC
CUGCGACAUUCUACCAACAGCAGAGGCCAAGAGAGCUAGCAAGGGCAACAAGACCUGCGGCUUC
GUGGACGAGAGAGGCCUGUACAAGAGCCUGAAGGGCGCCUGCAGACUGAAGCUGUGCGGCGUG
CUGGGCCUGAGACUGAUGGACGGCACCUGGGUGGCCAUGCAGACAAGCGACGAAACCAAGUGGU
GCCCCCGAUCAGCUGGUGAACCUGCACGACUUCAGAAGCGACGAGAUCCGAGCACCUGGUGGU
GGAGGAGCUGGUGAAGAAGAGAGAGGAGUGCCUGGACGCCUGGAGAGCAUCAUGACCACCAA
GAGCGUGUCCUUCAGAAGACUGAGCCACCUGAGAAAGCUGGUGCCCGGCUUCGGCAAGGCCUAC
ACCAUCUUCAACAAGACCCUGAUGGAGGCCGACGCCACUACAAAAGCGUGAGAACCUGGAACG
AGAUCAUCCCUAGCAAGGGCUGCCUGAAGGUGGGCGGCAGAUGCCACCCCAUGUGAACGGCGU
GUUCUUCAACGGCAUCAUCCUGGGCCCCGACGGCCACGUGCUGAUCCCCGAGAUCCAGAGCAGC
CUGCUGCAGCAGCACAUUGGAGCUGCUGAAGAGCAGCGUGAUCCCCCUGAUGCACCCCUUGGCCG
ACCCUAGCACCGUGUUAAGGAGGGCGACGAGGCCGAGGACUUCGUGGAGGUGCACCUGCCCGA
CGUGUACAAGCAGAUACGCGGCGUGGACCUGGGCCUGCCCAACUGGGGCAAGUACGUGCUGAUG
ACCGCCGGCGCCAUGAUCGGCCUGGUGCUGAUUCUUCAGCCUGAUGACCUGGUGCAGAAGAGCCA
ACAGACCCGAGAGCAAGCAGCGGAGCUUCGGCGGCACCGGCAGAAACGUGAGCGUGACAAGCCA
AAGCGGCAAGGUGAUCCCUAGCUGGGAGAGCUACAAGAGCGGCGGGCAGACAAGACUGUGAUA
AGAAUUCUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCAGCCCCUC
CUCCCCUCCUGCACCCGUACCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGCACUAGUAA
 AA
 AAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 18 (MRNA 18)

(G^{m7})ppp(A^{m2})UGGGGAUCCAAGCUUAGCUUGCUUGUUCUUUUUGCAGAAGCUCAGAAUAAACGCU
CAACUUUGGCGCCGCCACCAUGGUGAGCAAGGGCGAGGAGCUGUUCACCGGGGUGGUGCCCAUC
CUGGUCGAGCUGGACGGCGACGUAAACGGCCACAAGUUCAGCGUGUCCGGCGAGGGCGAGGGCG
AUGCCACCUACGGCAAGCUGACCCUGAAGUUAUCUGCACCACCGGCAAGCUGCCCGUGCCCUUG
GCCCACCCUCGUGACCACCCUGACCUACGGCGUGCAGUGCUUCAGCCGCUACCCCGACCAUGA
AGCAGCAGCAGCUUCUUAAGUCCGCCAUGCCCGAAGGCUACGUCCAGGAGCGCACCAUCUUCU
CAAGGACGACGGCAACUACAAGACCCGCGCCGAGGUGAAGUUCGAGGGCGACACCCUGGUGAAC
CGCAUCGAGCUGAAGGGCAUCGACUUAAGGAGGACGGCAACAUCUGGGGCAACAAGCUGGAGU
ACAACUACAACAGCCACAACGUCUAUAUCAUGGCCGACAAGCAGAGAAGACGGCAUCAAGGUGAA
CUUCAAGAUCGCCACAACAUCGAGGACGGCAGCGUGCAGCUCGCCGACCACUACCAGCAGAAC
ACCCCAUCGGCGACGGCCCCGUGCUGCUGCCCAGAACCAUACCUGAGCACCAGUCCGCCCU
GAGCAAAGACCCCAACGAGAAGCGCAUACAUGGUCCUGCUGGAGUUCGUGACCGCCCGGG
AUCACUCUGGCAUGGACGAGCUGUACAAGUGAUAAGAAUUCUAGGCUGGAGCCUCGGUGGCC
AUGCUUCUUGCCCCUUGGGCCUCCCCCAGCCCUCCUCCCUUCCUGCACCCGUACCCCGUGG

UCUUUGAAUAAAGUCUGAGUGGGCGGCACUAGUAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAA

SEQ ID NO: 19 (MRNA 19)

(G^{m7,3'OMe})ppp(A^{m2})UGGGGAUCCAAGCUUAGCUUGCUUGUUCUUUUUGCAGAAGCUCAGAAUAAAC
GCUCAACUUUGGCGCCGCCACCAUGGUGAGCAAGGGCGAGGAGCUGUUCACCGGGGUGGUGCCC
AUCCUGGUCGAGCUGGACGCGACGUAAACGGCCACAAGUUCAGCGUGUCCGGCGAGGGCGAGG
GCGAUGCCACCUACGGCAAGCUGACCCUGAAGUUCUUCUGCACCACCGGCAAGCUGCCCCGUGCC
CUGGCCCCACCCUCGUGACCACCCUGACCUACGGCGUGCAGUGCUUCAGCCGCUACCCCCGACCACA
UGAAGCAGCAGCAGCUUCUUCUUAAGUCCGCCAUGCCCCGAAGGCUACGUCCAGGAGCGCACCAUCUU
CUUCAAGGACGACGGCAACUACAAGACCCGCGCCGAGGUGAAGUUCGAGGGCGACACCCUGGUG
AACCGCAUCGAGCUGAAGGGCAUCGACUUAAGGAGGACGGCAACAUCUGGGGCAACAAGCUGG
AGUACAACUACAACAGCCACAACGUCUAUAUCAUGGCCGACAAGCAGAAGAACGGCAUCAAGGU
GAACUUAAGAUCGCCACAACAUCGAGGACGGCAGCGUGCAGCUCGCCGACCACUACCAGCAG
AACACCCCCAUCGGCGACGGCCCCGUGCUGCUGCCGACAACCACUACCUGAGCACCCAGUCCGC
CCUGAGCAAAGACCCCAACGAGAAGCGGAUCACAUGGUCCUGCUGGAGUUCGUGACCGCCGCC
GGGAUCACUCUCGGCAUGGACGAGCUGUACAAGUGAUAAAGAAUUCUAGGCUGGAGCCUCGGUG
GCCAUGCUUCUUGCCCCUUGGGCCUCCCCCAGCCCCUCCUCCCCUCCUGCACCCGUACCCCGG
UGGUCUUUGAAUAAAGUCUGAGUGGGCGGCACUAGUAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAA

SEQ ID NO: 20 (MRNA 20)

(G^{m7})ppp(A^{m2})UGGGGAUCCAAGCUUAGCUUGCUUGUUCUUUUUGCAGAAGCUCAGAAUAAACGC
UCAACUUUGGCGCCGCCACCAUGGUGAGCAAGGGCGAGGAGCUGUUCACCGGGGUGGUGCCCAU
CCUGGUCGAGCUGGACGCGACGUAAACGGCCACAAGUUCAGCGUGUCCGGCGAGGGCGAGGGC
GAUGCCACCUACGGCAAGCUGACCCUGAAGUUCUUCUGCACCACCGGCAAGCUGCCCGUGCCCU
GGCCCACCCUCGUGACCACCCUGACCUACGGCGUGCAGUGCUUCAGCCGCUACCCCCGACCACAUG
AAGCAGCAGCAGCUUCUUAAGUCCGCCAUGCCCCGAAGGCUACGUCCAGGAGCGCACCAUCUUCU
UCAAGGACGACGGCAACUACAAGACCCGCGCCGAGGUGAAGUUCGAGGGCGACACCCUGGUGAA
CCGCAUCGAGCUGAAGGGCAUCGACUUAAGGAGGACGGCAACAUCUGGGGCAACAAGCUGGAG
UACAACUACAACAGCCACAACGUCUAUAUCAUGGCCGACAAGCAGAAGAACGGCAUCAAGGUGA
ACUUAAGAUCGCCACAACAUCGAGGACGGCAGCGUGCAGCUCGCCGACCACUACCAGCAGAA
CACCCCCAUCGGCGACGGCCCCGUGCUGCUGCCGACAACCACUACCUGAGCACCCAGUCCGCC
UGAGCAAAGACCCCAACGAGAAGCGGAUCACAUGGUCCUGCUGGAGUUCGUGACCGCCGCCGG
GAUCACUCUCGGCAUGGACGAGCUGUACAAGUGAUAAAGAAUUCUAGGCUGGAGCCUCGGUGGC
CAUGCUUCUUGCCCCUUGGGCCUCCCCCAGCCCCUCCUCCCCUCCUGCACCCGUACCCCGUG
GUCUUUGAAUAAAGUCUGAGUGGGCGGCACUAGUAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAA

SEQ ID NO: 21 (MRNA 21)

(G^{m7})vppp(A^{m2})UGGGGAUCCAAGCUUAGCUUGCUUGUUCUUUUUGCAGAAGCUCAGAAUAAACGC
UCAACUUUGGCGCCGCCACCAUGGUGAGCAAGGGCGAGGAGCUGUUCACCGGGGUGGUGCCCAU
CCUGGUCGAGCUGGACGCGACGUAAACGGCCACAAGUUCAGCGUGUCCGGCGAGGGCGAGGGC
GAUGCCACCUACGGCAAGCUGACCCUGAAGUUCUUCUGCACCACCGGCAAGCUGCCCGUGCCCU
GGCCCACCCUCGUGACCACCCUGACCUACGGCGUGCAGUGCUUCAGCCGCUACCCCCGACCACAUG
AAGCAGCAGCAGCUUCUUAAGUCCGCCAUGCCCCGAAGGCUACGUCCAGGAGCGCACCAUCUUCU
UCAAGGACGACGGCAACUACAAGACCCGCGCCGAGGUGAAGUUCGAGGGCGACACCCUGGUGAA
CCGCAUCGAGCUGAAGGGCAUCGACUUAAGGAGGACGGCAACAUCUGGGGCAACAAGCUGGAG
UACAACUACAACAGCCACAACGUCUAUAUCAUGGCCGACAAGCAGAAGAACGGCAUCAAGGUGA
ACUUAAGAUCGCCACAACAUCGAGGACGGCAGCGUGCAGCUCGCCGACCACUACCAGCAGAA

CACCCCCAUCGGCGACGGCCCCGUGCUGCUGCCCGACAACCACUACCUGAGCACCCAGUCCGCC
 UGAGCAAAGACCCCAACGAGAAGCGCGAUCACAUGGUCCUGCUGGAGUUCGUGACCGCCGCCG
 GAUCACUCUCGGCAUGGACGAGCUGUACAAGUGAUAAAGAAUUCUAGGCUGGAGCCUCGGUGGC
CAUGCUUCUUGCCCCUUGGGCCUCCCCCAGCCCCUCCUCCCCUCCUGCACCCGUACCCCCGUG
GUCUUUGAAUAAAGUCUGAGUGGGCGGCACUAGUAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AA

SEQ ID NO: 22 (MRNA 22)

(G^{m7})ppp(A^{m2})UGGGGA¹CCCG²PGG³Y⁴Y⁵GG⁶YGA⁷Y⁸Y⁹Y¹⁰Y¹¹Y¹²Y¹³Y¹⁴Y¹⁵Y¹⁶Y¹⁷Y¹⁸Y¹⁹Y²⁰Y²¹Y²²Y²³Y²⁴Y²⁵Y²⁶Y²⁷Y²⁸Y²⁹Y³⁰Y³¹Y³²Y³³Y³⁴Y³⁵Y³⁶Y³⁷Y³⁸Y³⁹Y⁴⁰Y⁴¹Y⁴²Y⁴³Y⁴⁴Y⁴⁵Y⁴⁶Y⁴⁷Y⁴⁸Y⁴⁹Y⁵⁰Y⁵¹Y⁵²Y⁵³Y⁵⁴Y⁵⁵Y⁵⁶Y⁵⁷Y⁵⁸Y⁵⁹Y⁶⁰Y⁶¹Y⁶²Y⁶³Y⁶⁴Y⁶⁵Y⁶⁶Y⁶⁷Y⁶⁸Y⁶⁹Y⁷⁰Y⁷¹Y⁷²Y⁷³Y⁷⁴Y⁷⁵Y⁷⁶Y⁷⁷Y⁷⁸Y⁷⁹Y⁸⁰Y⁸¹Y⁸²Y⁸³Y⁸⁴Y⁸⁵Y⁸⁶Y⁸⁷Y⁸⁸Y⁸⁹Y⁹⁰Y⁹¹Y⁹²Y⁹³Y⁹⁴Y⁹⁵Y⁹⁶Y⁹⁷Y⁹⁸Y⁹⁹Y¹⁰⁰Y¹⁰¹Y¹⁰²Y¹⁰³Y¹⁰⁴Y¹⁰⁵Y¹⁰⁶Y¹⁰⁷Y¹⁰⁸Y¹⁰⁹Y¹¹⁰Y¹¹¹Y¹¹²Y¹¹³Y¹¹⁴Y¹¹⁵Y¹¹⁶Y¹¹⁷Y¹¹⁸Y¹¹⁹Y¹²⁰Y¹²¹Y¹²²Y¹²³Y¹²⁴Y¹²⁵Y¹²⁶Y¹²⁷Y¹²⁸Y¹²⁹Y¹³⁰Y¹³¹Y¹³²Y¹³³Y¹³⁴Y¹³⁵Y¹³⁶Y¹³⁷Y¹³⁸Y¹³⁹Y¹⁴⁰Y¹⁴¹Y¹⁴²Y¹⁴³Y¹⁴⁴Y¹⁴⁵Y¹⁴⁶Y¹⁴⁷Y¹⁴⁸Y¹⁴⁹Y¹⁵⁰Y¹⁵¹Y¹⁵²Y¹⁵³Y¹⁵⁴Y¹⁵⁵Y¹⁵⁶Y¹⁵⁷Y¹⁵⁸Y¹⁵⁹Y¹⁶⁰Y¹⁶¹Y¹⁶²Y¹⁶³Y¹⁶⁴Y¹⁶⁵Y¹⁶⁶Y¹⁶⁷Y¹⁶⁸Y¹⁶⁹Y¹⁷⁰Y¹⁷¹Y¹⁷²Y¹⁷³Y¹⁷⁴Y¹⁷⁵Y¹⁷⁶Y¹⁷⁷Y¹⁷⁸Y¹⁷⁹Y¹⁸⁰Y¹⁸¹Y¹⁸²Y¹⁸³Y¹⁸⁴Y¹⁸⁵Y¹⁸⁶Y¹⁸⁷Y¹⁸⁸Y¹⁸⁹Y¹⁹⁰Y¹⁹¹Y¹⁹²Y¹⁹³Y¹⁹⁴Y¹⁹⁵Y¹⁹⁶Y¹⁹⁷Y¹⁹⁸Y¹⁹⁹Y²⁰⁰Y²⁰¹Y²⁰²Y²⁰³Y²⁰⁴Y²⁰⁵Y²⁰⁶Y²⁰⁷Y²⁰⁸Y²⁰⁹Y²¹⁰Y²¹¹Y²¹²Y²¹³Y²¹⁴Y²¹⁵Y²¹⁶Y²¹⁷Y²¹⁸Y²¹⁹Y²²⁰Y²²¹Y²²²Y²²³Y²²⁴Y²²⁵Y²²⁶Y²²⁷Y²²⁸Y²²⁹Y²³⁰Y²³¹Y²³²Y²³³Y²³⁴Y²³⁵Y²³⁶Y²³⁷Y²³⁸Y²³⁹Y²⁴⁰Y²⁴¹Y²⁴²Y²⁴³Y²⁴⁴Y²⁴⁵Y²⁴⁶Y²⁴⁷Y²⁴⁸Y²⁴⁹Y²⁵⁰Y²⁵¹Y²⁵²Y²⁵³Y²⁵⁴Y²⁵⁵Y²⁵⁶Y²⁵⁷Y²⁵⁸Y²⁵⁹Y²⁶⁰Y²⁶¹Y²⁶²Y²⁶³Y²⁶⁴Y²⁶⁵Y²⁶⁶Y²⁶⁷Y²⁶⁸Y²⁶⁹Y²⁷⁰Y²⁷¹Y²⁷²Y²⁷³Y²⁷⁴Y²⁷⁵Y²⁷⁶Y²⁷⁷Y²⁷⁸Y²⁷⁹Y²⁸⁰Y²⁸¹Y²⁸²Y²⁸³Y²⁸⁴Y²⁸⁵Y²⁸⁶Y²⁸⁷Y²⁸⁸Y²⁸⁹Y²⁹⁰Y²⁹¹Y²⁹²Y²⁹³Y²⁹⁴Y²⁹⁵Y²⁹⁶Y²⁹⁷Y²⁹⁸Y²⁹⁹Y³⁰⁰Y³⁰¹Y³⁰²Y³⁰³Y³⁰⁴Y³⁰⁵Y³⁰⁶Y³⁰⁷Y³⁰⁸Y³⁰⁹Y³¹⁰Y³¹¹Y³¹²Y³¹³Y³¹⁴Y³¹⁵Y³¹⁶Y³¹⁷Y³¹⁸Y³¹⁹Y³²⁰Y³²¹Y³²²Y³²³Y³²⁴Y³²⁵Y³²⁶Y³²⁷Y³²⁸Y³²⁹Y³³⁰Y³³¹Y³³²Y³³³Y³³⁴Y³³⁵Y³³⁶Y³³⁷Y³³⁸Y³³⁹Y³⁴⁰Y³⁴¹Y³⁴²Y³⁴³Y³⁴⁴Y³⁴⁵Y³⁴⁶Y³⁴⁷Y³⁴⁸Y³⁴⁹Y³⁵⁰Y³⁵¹Y³⁵²Y³⁵³Y³⁵⁴Y³⁵⁵Y³⁵⁶Y³⁵⁷Y³⁵⁸Y³⁵⁹Y³⁶⁰Y³⁶¹Y³⁶²Y³⁶³Y³⁶⁴Y³⁶⁵Y³⁶⁶Y³⁶⁷Y³⁶⁸Y³⁶⁹Y³⁷⁰Y³⁷¹Y³⁷²Y³⁷³Y³⁷⁴Y³⁷⁵Y³⁷⁶Y³⁷⁷Y³⁷⁸Y³⁷⁹Y³⁸⁰Y³⁸¹Y³⁸²Y³⁸³Y³⁸⁴Y³⁸⁵Y³⁸⁶Y³⁸⁷Y³⁸⁸Y³⁸⁹Y³⁹⁰Y³⁹¹Y³⁹²Y³⁹³Y³⁹⁴Y³⁹⁵Y³⁹⁶Y³⁹⁷Y³⁹⁸Y³⁹⁹Y⁴⁰⁰Y⁴⁰¹Y⁴⁰²Y⁴⁰³Y⁴⁰⁴Y⁴⁰⁵Y⁴⁰⁶Y⁴⁰⁷Y⁴⁰⁸Y⁴⁰⁹Y⁴¹⁰Y⁴¹¹Y⁴¹²Y⁴¹³Y⁴¹⁴Y⁴¹⁵Y⁴¹⁶Y⁴¹⁷Y⁴¹⁸Y⁴¹⁹Y⁴²⁰Y⁴²¹Y⁴²²Y⁴²³Y⁴²⁴Y⁴²⁵Y⁴²⁶Y⁴²⁷Y⁴²⁸Y⁴²⁹Y⁴³⁰Y⁴³¹Y⁴³²Y⁴³³Y⁴³⁴Y⁴³⁵Y⁴³⁶Y⁴³⁷Y⁴³⁸Y⁴³⁹Y⁴⁴⁰Y⁴⁴¹Y⁴⁴²Y⁴⁴³Y⁴⁴⁴Y⁴⁴⁵Y⁴⁴⁶Y⁴⁴⁷Y⁴⁴⁸Y⁴⁴⁹Y⁴⁵⁰Y⁴⁵¹Y⁴⁵²Y⁴⁵³Y⁴⁵⁴Y⁴⁵⁵Y⁴⁵⁶Y⁴⁵⁷Y⁴⁵⁸Y⁴⁵⁹Y⁴⁶⁰Y⁴⁶¹Y⁴⁶²Y⁴⁶³Y⁴⁶⁴Y⁴⁶⁵Y⁴⁶⁶Y⁴⁶⁷Y⁴⁶⁸Y⁴⁶⁹Y⁴⁷⁰Y⁴⁷¹Y⁴⁷²Y⁴⁷³Y⁴⁷⁴Y⁴⁷⁵Y⁴⁷⁶Y⁴⁷⁷Y⁴⁷⁸Y⁴⁷⁹Y⁴⁸⁰Y⁴⁸¹Y⁴⁸²Y⁴⁸³Y⁴⁸⁴Y⁴⁸⁵Y⁴⁸⁶Y⁴⁸⁷Y⁴⁸⁸Y⁴⁸⁹Y⁴⁹⁰Y⁴⁹¹Y⁴⁹²Y⁴⁹³Y⁴⁹⁴Y⁴⁹⁵Y⁴⁹⁶Y⁴⁹⁷Y⁴⁹⁸Y⁴⁹⁹Y⁵⁰⁰Y⁵⁰¹Y⁵⁰²Y⁵⁰³Y⁵⁰⁴Y⁵⁰⁵Y⁵⁰⁶Y⁵⁰⁷Y⁵⁰⁸Y⁵⁰⁹Y⁵¹⁰Y⁵¹¹Y⁵¹²Y⁵¹³Y⁵¹⁴Y⁵¹⁵Y⁵¹⁶Y⁵¹⁷Y⁵¹⁸Y⁵¹⁹Y⁵²⁰Y⁵²¹Y⁵²²Y⁵²³Y⁵²⁴Y⁵²⁵Y⁵²⁶Y⁵²⁷Y⁵²⁸Y⁵²⁹Y⁵³⁰Y⁵³¹Y⁵³²Y⁵³³Y⁵³⁴Y⁵³⁵Y⁵³⁶Y⁵³⁷Y⁵³⁸Y⁵³⁹Y⁵⁴⁰Y⁵⁴¹Y⁵⁴²Y⁵⁴³Y⁵⁴⁴Y⁵⁴⁵Y⁵⁴⁶Y⁵⁴⁷Y⁵⁴⁸Y⁵⁴⁹Y⁵⁵⁰Y⁵⁵¹Y⁵⁵²Y⁵⁵³Y⁵⁵⁴Y⁵⁵⁵Y⁵⁵⁶Y⁵⁵⁷Y⁵⁵⁸Y⁵⁵⁹Y⁵⁶⁰Y⁵⁶¹Y⁵⁶²Y⁵⁶³Y⁵⁶⁴Y⁵⁶⁵Y⁵⁶⁶Y⁵⁶⁷Y⁵⁶⁸Y⁵⁶⁹Y⁵⁷⁰Y⁵⁷¹Y⁵⁷²Y⁵⁷³Y⁵⁷⁴Y⁵⁷⁵Y⁵⁷⁶Y⁵⁷⁷Y⁵⁷⁸Y⁵⁷⁹Y⁵⁸⁰Y⁵⁸¹Y⁵⁸²Y⁵⁸³Y⁵⁸⁴Y⁵⁸⁵Y⁵⁸⁶Y⁵⁸⁷Y⁵⁸⁸Y⁵⁸⁹Y⁵⁹⁰Y⁵⁹¹Y⁵⁹²Y⁵⁹³Y⁵⁹⁴Y⁵⁹⁵Y⁵⁹⁶Y⁵⁹⁷Y⁵⁹⁸Y⁵⁹⁹Y⁶⁰⁰Y⁶⁰¹Y⁶⁰²Y⁶⁰³Y⁶⁰⁴Y⁶⁰⁵Y⁶⁰⁶Y⁶⁰⁷Y⁶⁰⁸Y⁶⁰⁹Y⁶¹⁰Y⁶¹¹Y⁶¹²Y⁶¹³Y⁶¹⁴Y⁶¹⁵Y⁶¹⁶Y⁶¹⁷Y⁶¹⁸Y⁶¹⁹Y⁶²⁰Y⁶²¹Y⁶²²Y⁶²³Y⁶²⁴Y⁶²⁵Y⁶²⁶Y⁶²⁷Y⁶²⁸Y⁶²⁹Y⁶³⁰Y⁶³¹Y⁶³²Y⁶³³Y⁶³⁴Y⁶³⁵Y⁶³⁶Y⁶³⁷Y⁶³⁸Y⁶³⁹Y⁶⁴⁰Y⁶⁴¹Y⁶⁴²Y⁶⁴³Y⁶⁴⁴Y⁶⁴⁵Y⁶⁴⁶Y⁶⁴⁷Y⁶⁴⁸Y⁶⁴⁹Y⁶⁵⁰Y⁶⁵¹Y⁶⁵²Y⁶⁵³Y⁶⁵⁴Y⁶⁵⁵Y⁶⁵⁶Y⁶⁵⁷Y⁶⁵⁸Y⁶⁵⁹Y⁶⁶⁰Y⁶⁶¹Y⁶⁶²Y⁶⁶³Y⁶⁶⁴Y⁶⁶⁵Y⁶⁶⁶Y⁶⁶⁷Y⁶⁶⁸Y⁶⁶⁹Y⁶⁷⁰Y⁶⁷¹Y⁶⁷²Y⁶⁷³Y⁶⁷⁴Y⁶⁷⁵Y⁶⁷⁶Y⁶⁷⁷Y⁶⁷⁸Y⁶⁷⁹Y⁶⁸⁰Y⁶⁸¹Y⁶⁸²Y⁶⁸³Y⁶⁸⁴Y⁶⁸⁵Y⁶⁸⁶Y⁶⁸⁷Y⁶⁸⁸Y⁶⁸⁹Y⁶⁹⁰Y⁶⁹¹Y⁶⁹²Y⁶⁹³Y⁶⁹⁴Y⁶⁹⁵Y⁶⁹⁶Y⁶⁹⁷Y⁶⁹⁸Y⁶⁹⁹Y⁷⁰⁰Y⁷⁰¹Y⁷⁰²Y⁷⁰³Y⁷⁰⁴Y⁷⁰⁵Y⁷⁰⁶Y⁷⁰⁷Y⁷⁰⁸Y⁷⁰⁹Y⁷¹⁰Y⁷¹¹Y⁷¹²Y⁷¹³Y⁷¹⁴Y⁷¹⁵Y⁷¹⁶Y⁷¹⁷Y⁷¹⁸Y⁷¹⁹Y⁷²⁰Y⁷²¹Y⁷²²Y⁷²³Y⁷²⁴Y⁷²⁵Y⁷²⁶Y⁷²⁷Y⁷²⁸Y⁷²⁹Y⁷³⁰Y⁷³¹Y⁷³²Y⁷³³Y⁷³⁴Y⁷³⁵Y⁷³⁶Y⁷³⁷Y⁷³⁸Y⁷³⁹Y⁷⁴⁰Y⁷⁴¹Y⁷⁴²Y⁷⁴³Y⁷⁴⁴Y⁷⁴⁵Y⁷⁴⁶Y⁷⁴⁷Y⁷⁴⁸Y⁷⁴⁹Y⁷⁵⁰Y⁷⁵¹Y⁷⁵²Y⁷⁵³Y⁷⁵⁴Y⁷⁵⁵Y⁷⁵⁶Y⁷⁵⁷Y⁷⁵⁸Y⁷⁵⁹Y⁷⁶⁰Y⁷⁶¹Y⁷⁶²Y⁷⁶³Y⁷⁶⁴Y⁷⁶⁵Y⁷⁶⁶Y⁷⁶⁷Y⁷⁶⁸Y⁷⁶⁹Y⁷⁷⁰Y⁷⁷¹Y⁷⁷²Y⁷⁷³Y⁷⁷⁴Y⁷⁷⁵Y⁷⁷⁶Y⁷⁷⁷Y⁷⁷⁸Y⁷⁷⁹Y⁷⁸⁰Y⁷⁸¹Y⁷⁸²Y⁷⁸³Y⁷⁸⁴Y⁷⁸⁵Y⁷⁸⁶Y⁷⁸⁷Y⁷⁸⁸Y⁷⁸⁹Y⁷⁹⁰Y⁷⁹¹Y⁷⁹²Y⁷⁹³Y⁷⁹⁴Y⁷⁹⁵Y⁷⁹⁶Y⁷⁹⁷Y⁷⁹⁸Y⁷⁹⁹Y⁸⁰⁰Y⁸⁰¹Y⁸⁰²Y⁸⁰³Y⁸⁰⁴Y⁸⁰⁵Y⁸⁰⁶Y⁸⁰⁷Y⁸⁰⁸Y⁸⁰⁹Y⁸¹⁰Y⁸¹¹Y⁸¹²Y⁸¹³Y⁸¹⁴Y⁸¹⁵Y⁸¹⁶Y⁸¹⁷Y⁸¹⁸Y⁸¹⁹Y⁸²⁰Y⁸²¹Y⁸²²Y⁸²³Y⁸²⁴Y⁸²⁵Y⁸²⁶Y⁸²⁷Y⁸²⁸Y⁸²⁹Y⁸³⁰Y⁸³¹Y⁸³²Y⁸³³Y⁸³⁴Y⁸³⁵Y⁸³⁶Y⁸³⁷Y⁸³⁸Y⁸³⁹Y⁸⁴⁰Y⁸⁴¹Y⁸⁴²Y⁸⁴³Y⁸⁴⁴Y⁸⁴⁵Y⁸⁴⁶Y⁸⁴⁷Y⁸⁴⁸Y⁸⁴⁹Y⁸⁵⁰Y⁸⁵¹Y⁸⁵²Y⁸⁵³Y⁸⁵⁴Y⁸⁵⁵Y⁸⁵⁶Y⁸⁵⁷Y⁸⁵⁸Y⁸⁵⁹Y⁸⁶⁰Y⁸⁶¹Y⁸⁶²Y⁸⁶³Y⁸⁶⁴Y⁸⁶⁵Y⁸⁶⁶Y⁸⁶⁷Y⁸⁶⁸Y⁸⁶⁹Y⁸⁷⁰Y⁸⁷¹Y⁸⁷²Y⁸⁷³Y⁸⁷⁴Y⁸⁷⁵Y⁸⁷⁶Y⁸⁷⁷Y⁸⁷⁸Y⁸⁷⁹Y⁸⁸⁰Y⁸⁸¹Y⁸⁸²Y⁸⁸³Y⁸⁸⁴Y⁸⁸⁵Y⁸⁸⁶Y⁸⁸⁷Y⁸⁸⁸Y⁸⁸⁹Y⁸⁹⁰Y⁸⁹¹Y⁸⁹²Y⁸⁹³Y⁸⁹⁴Y⁸⁹⁵Y⁸⁹⁶Y⁸⁹⁷Y⁸⁹⁸Y⁸⁹⁹Y⁹⁰⁰Y⁹⁰¹Y⁹⁰²Y⁹⁰³Y⁹⁰⁴Y⁹⁰⁵Y⁹⁰⁶Y⁹⁰⁷Y⁹⁰⁸Y⁹⁰⁹Y⁹¹⁰Y⁹¹¹Y⁹¹²Y⁹¹³Y⁹¹⁴Y⁹¹⁵Y⁹¹⁶Y⁹¹⁷Y⁹¹⁸Y⁹¹⁹Y⁹²⁰Y⁹²¹Y⁹²²Y⁹²³Y⁹²⁴Y⁹²⁵Y⁹²⁶Y⁹²⁷Y⁹²⁸Y⁹²⁹Y⁹³⁰Y⁹³¹Y⁹³²Y⁹³³Y⁹³⁴Y⁹³⁵Y⁹³⁶Y⁹³⁷Y⁹³⁸Y⁹³⁹Y⁹⁴⁰Y⁹⁴¹Y⁹⁴²Y⁹⁴³Y⁹⁴⁴Y⁹⁴⁵Y⁹⁴⁶Y⁹⁴⁷Y⁹⁴⁸Y⁹⁴⁹Y⁹⁵⁰Y⁹⁵¹Y⁹⁵²Y⁹⁵³Y⁹⁵⁴Y⁹⁵⁵Y⁹⁵⁶Y⁹⁵⁷Y⁹⁵⁸Y⁹⁵⁹Y⁹⁶⁰Y⁹⁶¹Y⁹⁶²Y⁹⁶³Y⁹⁶⁴Y⁹⁶⁵Y⁹⁶⁶Y⁹⁶⁷Y⁹⁶⁸Y⁹⁶⁹Y⁹⁷⁰Y⁹⁷¹Y⁹⁷²Y⁹⁷³Y⁹⁷⁴Y⁹⁷⁵Y⁹⁷⁶Y⁹⁷⁷Y⁹⁷⁸Y⁹⁷⁹Y⁹⁸⁰Y⁹⁸¹Y⁹⁸²Y⁹⁸³Y⁹⁸⁴Y⁹⁸⁵Y⁹⁸⁶Y⁹⁸⁷Y⁹⁸⁸Y⁹⁸⁹Y⁹⁹⁰Y⁹⁹¹Y⁹⁹²Y⁹⁹³Y⁹⁹⁴Y⁹⁹⁵Y⁹⁹⁶Y⁹⁹⁷Y⁹⁹⁸Y⁹⁹⁹Y¹⁰⁰⁰Y¹⁰⁰¹Y¹⁰⁰²Y¹⁰⁰³Y¹⁰⁰⁴Y¹⁰⁰⁵Y¹⁰⁰⁶Y¹⁰⁰⁷Y¹⁰⁰⁸Y¹⁰⁰⁹Y¹⁰¹⁰Y¹⁰¹¹Y¹⁰¹²Y¹⁰¹³Y¹⁰¹⁴Y¹⁰¹⁵Y¹⁰¹⁶Y¹⁰¹⁷Y¹⁰¹⁸Y¹⁰¹⁹Y¹⁰²⁰Y¹⁰²¹Y¹⁰²²Y¹⁰²³Y¹⁰²⁴Y¹⁰²⁵Y¹⁰²⁶Y¹⁰²⁷Y¹⁰²⁸Y¹⁰²⁹Y¹⁰³⁰Y¹⁰³¹Y¹⁰³²Y¹⁰³³Y¹⁰³⁴Y¹⁰³⁵Y¹⁰³⁶Y¹⁰³⁷Y¹⁰³⁸Y¹⁰³⁹Y¹⁰⁴⁰Y¹⁰⁴¹Y¹⁰⁴²Y¹⁰⁴³Y¹⁰⁴⁴Y¹⁰⁴⁵Y¹⁰⁴⁶Y¹⁰⁴⁷Y¹⁰⁴⁸Y¹⁰⁴⁹Y¹⁰⁵⁰Y¹⁰⁵¹Y¹⁰⁵²Y¹⁰⁵³Y¹⁰⁵⁴Y¹⁰⁵⁵Y¹⁰⁵⁶Y¹⁰⁵⁷Y¹⁰⁵⁸Y¹⁰⁵⁹Y¹⁰⁶⁰Y¹⁰⁶¹Y¹⁰⁶²Y¹⁰⁶³Y¹⁰⁶⁴Y¹⁰⁶⁵Y¹⁰⁶⁶Y¹⁰⁶⁷Y¹⁰⁶⁸Y¹⁰⁶⁹Y¹⁰⁷⁰Y¹⁰⁷¹Y¹⁰⁷²Y¹⁰⁷³Y¹⁰⁷⁴Y¹⁰⁷⁵Y¹⁰⁷⁶Y¹⁰⁷⁷Y¹⁰⁷⁸Y¹⁰⁷⁹Y¹⁰⁸⁰Y¹⁰⁸¹Y¹⁰⁸²Y¹⁰⁸³Y¹⁰⁸⁴Y¹⁰⁸⁵Y¹⁰⁸⁶Y¹⁰⁸⁷Y¹⁰⁸⁸Y¹⁰⁸⁹Y¹⁰⁹⁰Y¹⁰⁹¹Y¹⁰⁹²Y¹⁰⁹³Y¹⁰⁹⁴Y¹⁰⁹⁵Y¹⁰⁹⁶Y¹⁰⁹⁷Y¹⁰⁹⁸Y¹⁰⁹⁹Y¹¹⁰⁰Y¹¹⁰¹Y¹¹⁰²Y¹¹⁰³Y¹¹⁰⁴Y¹¹⁰⁵Y¹¹⁰⁶Y¹¹⁰⁷Y¹¹⁰⁸Y¹¹⁰⁹Y¹¹¹⁰Y¹¹¹¹Y¹¹¹²Y¹¹¹³Y¹¹¹⁴Y¹¹¹⁵Y¹¹¹⁶Y¹¹¹⁷Y¹¹¹⁸Y¹¹¹⁹Y¹¹²⁰Y¹¹²¹Y¹¹²²Y¹¹²³Y¹¹²⁴Y¹¹²⁵Y¹¹²⁶Y¹¹²⁷Y¹¹²⁸Y¹¹²⁹Y¹¹³⁰Y¹¹³¹Y¹¹³²Y¹¹³³Y¹¹³⁴Y¹¹³⁵Y¹¹³⁶Y¹¹³⁷Y¹¹³⁸Y¹¹³⁹Y¹¹⁴⁰Y¹¹⁴¹Y¹¹⁴²Y¹¹⁴³Y¹¹⁴⁴Y¹¹⁴⁵Y¹¹⁴⁶Y¹¹⁴⁷Y¹¹⁴⁸Y¹¹⁴⁹Y¹¹⁵⁰Y¹¹⁵¹Y¹¹⁵²Y¹¹⁵³Y¹¹⁵⁴Y¹¹⁵⁵Y¹¹⁵⁶Y¹¹⁵⁷Y¹¹⁵⁸Y¹¹⁵⁹Y¹¹⁶⁰Y¹¹⁶¹Y¹¹⁶²Y¹¹⁶³Y¹¹⁶⁴Y¹¹⁶⁵Y¹¹⁶⁶Y¹¹⁶⁷Y¹¹⁶⁸Y¹¹⁶⁹Y¹¹⁷⁰Y¹¹⁷¹Y¹¹⁷²Y¹¹⁷³Y¹¹⁷⁴Y¹¹⁷⁵Y¹¹⁷⁶Y¹¹⁷⁷Y¹¹⁷⁸Y¹¹⁷⁹Y¹¹⁸⁰Y¹¹⁸¹Y¹¹⁸²Y¹¹⁸³Y¹¹⁸⁴Y¹¹⁸⁵Y¹¹⁸⁶Y¹¹⁸⁷Y¹¹⁸⁸Y¹¹

ΨGAGCAACAACGΨGCAGATCGΨGAGACAGCAGΨCTΨACAGCAΨCAΨGΨGCAΨCAΨCAAGGAGG
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 ΨGCACACΨΨCΨCCCCΨGΨGCACGACCAAΨACGAAGGAGGGCAGCAACAΨCΨGΨCΨGACCCGGAC
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 GΨACAGCΨΨCCAACAAGAACAGGGGCAΨCAΨCAAGACCΨΨCΨΨAAΨGGGΨGCGACΨACGΨCA
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 CACGACGAΨCAΨCAΨCΨGΨGAΨCAΨΨGΨGAΨCCΨGCGΨCAGCCΨGAΨCGCCGΨGGGGCΨGCGΨCCΨ
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 AΨCGCΨΨΨCAGCAACΨGAΨAAGAAΨΨCGΨGCAAAACΨCGAΨCACΨAGCΨCΨGCGΨGAΨGΨGGCA
GAAGCGAAGGGAACCAGGΨΨΨGCAAAAGΨAACΨGΨGGΨGAΨGGAAAΨGΨGΨΨAGCCΨCAGACA
CΨACΨGAGGΨGGΨΨCΨΨΨCΨAΨCCΨAGΨACAGΨCACΨAGΨAAAAAAAAAAAAAAAAAAAAAAAAA
 AA

SEQ ID NO: 24 (MRNA 24)

(G^{m7})ppp(A^{m2})ΨGGGGAΨCCGCGΨGGΨΨΨΨGGΨGAΨΨΨΨGΨCΨGAΨΨGCAGCCACCAΨGGAGCΨG
 CΨGAΨCCΨCAAGGCCAACGCCAΨCACCACCAΨΨCΨGACAGCCGΨGACGΨΨCΨGΨΨΨΨGCCAGCG
 GGCAGAAACΨACCAGGAGΨΨCΨACCAGAGCACCΨGΨΨCΨGCCGΨGΨCCAAGGGCΨACCΨGG
 GCGCCCΨGCGCACCCGGGΨGGΨACACCΨCCGΨGAΨCACCACΨGAGCΨGΨCΨAACAΨCAAGGAGAΨ
 ΨAAGΨGCAACGGCACΨGACGCCAAGGΨGAAGCΨGAΨCAAGCAGGAGCΨGGACAAGΨACAAGAA
 CGCCGΨGACΨGACCΨGCAGCΨGCGΨCAΨGCAGAGCACCCCGCCACCGGGAGΨGGCAGCGCCAΨC
 ΨGCAGCGGGCΨGGCCGΨCΨGCAAGGΨGCGΨGCACCΨCGAGGGCGAGGΨGAAΨAAGAΨCAAGAGΨ
 GCΨCΨGCGΨGAGCACΨAACAAGGCΨGΨGGΨCAGΨCΨGΨCAAAΨGGGGΨGAGCGΨGCGΨGACCΨΨC
 AAGGΨGCGΨGGACCΨGAAGAAΨΨACAΨΨGACAAGCAGCΨGCGΨGCCΨAΨΨCΨGAAΨAAGCAGAGC
 ΨGCAGCAΨCCCCAACAAΨΨGAGACAGΨΨAΨCGAGΨΨΨCAGCAGAAGAACAACAGGCΨGCGΨΨGAG
 AΨCACACGGGAGΨΨCAGCGΨGAACGCGΨGGGGΨGACCACΨCCCGΨGΨCCACΨΨACAΨGCGΨGACCA
 ACΨCAGAGCΨGCGΨGΨCCCΨGAΨCAACGACAΨGCCΨAΨΨACAAACGACCAGAAGAAGΨΨGAΨGA
 GCAACAACGΨGCAGAΨCGΨGAGACAGCAGΨCΨΨACAGCAΨCAΨGΨGCAΨCAΨCAAGGAGGAGG
 ΨGCGΨGGCGΨACGΨGGΨGCAGCΨGCCCCΨGΨAΨGGGGΨCAΨCGACACCCΨΨGΨΨGGAAGCΨGC
 ACACΨΨCΨCCCCΨGΨGCACGACCAAAΨACGAAGGAGGGCAGCAACAΨCΨGΨCΨGACCCGGACAGA
 ΨCGGGGCGΨGGΨAΨΨGCGACAAΨGCCGGCΨCCGΨGΨCCΨΨCΨΨCCCCAGGCΨGAGACΨΨGCAA
 GGΨGCAGAGΨAACC GGΨGΨCΨGCGACACCAΨGAACΨCCCGACΨCΨGCCCΨCCGAGGΨGAACC
 ΨGΨGCAACGΨGGACAΨΨΨΨCAACCCCAAGΨACGAΨΨGCAAGAΨCAΨGACΨAGCAAGACCGACG
 ΨGΨCΨΨCΨAGCGΨGAΨΨACCAGCCΨGGGGGCGAΨΨGΨGΨCGΨGCGΨACGGGAAGACGAAGΨGΨA
 CAGCΨΨCCAACAAGAACAGGGGCAΨCAΨCAAGACCΨΨCΨΨAAΨGGGΨGCGACΨACGΨCAGCA
 AΨAAGGGGCGΨCGACACCGΨCAGCGΨGGGGAACACCΨΨGΨACΨGCGΨGAACAAGCAGGAGGGGC
 AGAGCCΨGΨACGΨGAAGGGGGAGCCCAΨΨAΨCAACΨΨCΨACGACCCCCΨGGΨGΨΨCCCCAGCGA
 CGAGΨΨCGACGCCΨCΨAΨCAGCCAGGΨGAACGAGAAGAΨCAAΨCAGΨCCCΨGGCCΨΨCAΨΨAG
 GAAGΨCΨGAΨGAGCΨGCGΨGCACAACGΨGAACGCCGGGAAGAGCACΨACGAAΨAΨCAΨGAΨCAC
 GACGAΨCAΨCAΨCGΨGAΨCAΨΨGΨGAΨCCΨGCGΨCAGCCΨGAΨCGCCGΨGGGGCΨGCGΨCCΨGΨA
 CΨGCAAGGCCAGGAGCACCCCGΨGACCCΨGΨCCAAGGAΨCAGCΨGAGCGGGAΨCAACAAΨAΨC
 GCΨΨΨCAGCAACΨGAΨAAGAAΨΨCGΨGCAAAACΨCGAΨCACΨAGCΨCΨGCGΨGAΨGΨGGCAGAA
GCGAAGGGAACCAGGΨΨΨGCAAAAGΨAACΨGΨGGΨGAΨGGAAAΨGΨGΨΨAGCCΨCAGACACΨA
CΨGAGGΨGGΨΨCΨΨΨCΨAΨCCΨAGΨACAGΨCACΨAGΨAAAAAAAAAAAAAAAAAAAAAAAAA
 AA

SEQ ID NO: 25 (MRNA 25)

(G^{m7})ppp(A^{m2})UGGGGAΨCCCΨGAACΨGGGΨΨAGGΨGCCGCΨGΨΨGCΨGCΨCGΨGΨΨGAAΨCΨAG
 AACCGΨAGCCAGACGCCACCAΨGGACΨGGACCΨGGAΨΨCΨGΨΨCCΨGGΨGGCCGCCGCCACAG
 GGΨGCACAGCΨCΨGΨCCΨGAGGΨACGACGACΨΨCCACAΨCGAΨGAGGACAAGCΨGGACACCAAC
 AGCGΨGΨACGAGCCCCΨACΨACCACAGCGACCACGCCGAGΨCCΨCCΨGGGΨGAAΨAGGGGCGAGΨ
 CΨΨCCAGGAAGGCCΨAΨGACCACAAΨAGCCCCΨACAΨCΨGGCCCCGGAACGACΨACGACGGCΨΨ
 CCΨGGAGAACGCCACGAGCACCACGGGGΨGΨACAACCAGGGCCCGCGGAΨCGAΨΨCCGGGGAG
 AGGCΨGAΨGCAGCCΨACCCAGAΨGΨCCGCCCAGGAGGACCΨGGGCGACGAΨACCGGCAΨΨCAGC
 ΨGAΨCCCCACCCΨGAAΨGGGGACGAΨCGGCACAAGAΨCGΨGAAΨGΨGGAΨCAGCGCCAGΨACG
 GCGAΨGΨGΨΨCAAGGGCGACCΨGAAΨCCCAAGCCCCAGGGCCAGCGGCΨGAΨCGAGGΨGAGCG
 ΨGGAGGAGAAΨCACCCΨΨΨACCCΨGCGGGCCCCCAΨΨCAGAGGAΨCΨACGGGGΨGCGGΨACA
 CCGAAACCΨGGAGCΨΨCΨGCCCAGCCΨGACCΨGΨACCGGCGACGCCGCCCGCCCAΨCCAGCA
 CAΨCΨGCCΨGAAGCAΨACCACCΨGCΨΨCCAGGACGΨGGΨGGΨGGACGΨGGACΨGCGCCGAGAA
 ΨACCAAGGAGGAΨCAGCΨGGCAGAGAΨCAGCΨACCGGΨΨΨCAGGGGAAGAAGGAGGCCGACCA
 GCCΨΨGGAΨCGΨGGΨGAACACCAGCACCCΨGΨΨCGACGAGCΨGGAGCΨGGACCCΨCCCGAGAΨC
 GAGCCCCGGCΨGΨGAAGGΨGCΨGAGGACCGAGAAGCAGΨACCΨGGGGGΨGΨACAΨCΨGGAAC
 AΨGCGGGGGAGCGAΨGGCACCAGCACAΨACGCCACCΨΨCCΨGGΨGACCΨGGAAGGGGGACGAG
 AAGACΨCGGAACCCCCACCCΨGCΨGΨGACCCACAGCCCAGGGGCGCΨGAGΨΨCCACAΨGΨGGA
 ACΨACCAΨAGCCACGΨGΨΨCAGCGΨGGGGGACACCΨΨCΨCCCΨGGCΨAΨGCACCΨCGAGΨACAA
 GAΨCCACAGAGGCCCCΨΨΨGAΨCΨGCΨGΨGGAGΨGGCΨGΨACGΨGGCCCAΨCGACCCACCΨGΨ
 CAGCCΨAΨGAGGCΨGΨACAGCACCΨGCCΨGΨACCACCCCAACGCCCCACAGΨGCCΨGΨCCCACA
 ΨGAACAGCGGGΨGCACCΨΨΨCACCAGCCCCACCΨGGCCACAGAGGGΨGGCCΨCCACCΨGΨACCA
 GAAΨΨGCGAGCACGCCGACAAΨΨACACCGCCΨACΨGΨCΨGGGGAΨCAGCCACAΨGGAGCCCAGC
 ΨΨCGGGCΨGAΨCCΨGCACGACGGGGGGACCAACCΨGAAGΨΨCGΨGGACACCCΨGAGAGCCΨGA
 GCGGGCΨGΨACGΨGΨΨCGΨGGΨGΨACΨΨΨAACGGGCACGΨGGAGGCCGΨGGCCΨACACCΨGG
 ΨGΨCCACCΨGGACCACΨΨCGΨGAACGCCAΨCGAGGAGAGGGGCΨΨCCΨCCΨACCGCCGGGA
 GCCCCCΨGCCACCACCAAGCCCAAGGAGAΨCACCCCGΨGAACCCCGGCACCAGCCCCΨGAΨCC
 GGΨACGCCGCCΨGGACAGGCGGGCΨGGCΨGCCGΨGGΨGCΨGCΨGΨGCCΨGGΨGAΨCΨΨCCΨGA
 ΨCΨGCACCGCCAAGCGGAΨGCGGGΨGAAGGCCGCCAGGGΨGGACAAGAGCCCCΨACAAΨCAGAG
 CAΨGΨACGGGGCΨGGGCΨGCCΨGAΨAAGAAΨΨCΨAGGCΨGGAGCCΨCGGΨGGCCAΨGCΨΨCΨ
 ΨGCCCCΨΨGGGCCΨCCCCCAGCCCCΨCCΨCCCCΨCCΨGCACCCGΨACCCCGΨGGCΨΨΨGAA
 ΨAAAGΨCΨGAGΨGGGCGGCACΨAGΨAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AA

SEQ ID NO: 26 (MRNA 26)

(G^{m7,3'OMe})ppp(A^{m2})UGGGGAΨCCCΨGAACΨGGGΨΨAGGΨGCCGCΨGΨΨGCΨGCΨCGΨGΨΨGAAΨC
 ΨAGAACCΨΨAGCCAGACGCCACCAΨGGACΨGGACCΨGGAΨΨCΨGΨΨCCΨGGΨGGCCGCCGCCAC
 CAGGGΨGCACAGCΨCΨGΨCCΨGAGGΨACGACGACΨΨCCACAΨCGAΨGAGGACAAGCΨGGACACC
 AACAGCGΨGΨACGAGCCCCΨACΨACCACAGCGACCACGCCGAGΨCCΨCCΨGGGΨGAAΨAGGGGCG
 AGΨCΨΨCCAGGAAGGCCΨAΨGACCACAAΨAGCCCCΨACAΨCΨGGCCCCGGAACGACΨACGACGG
 CΨΨCCΨGGAGAACGCCACGAGCACCACGGGGΨGΨACAACCAGGGCCCGCGGGAΨCGAΨΨCCGGG
 GAGAGGCΨGAΨGCAGCCΨACCCAGAΨGΨCCGCCCAGGAGGACCΨGGGCGACGAΨACCGGCAΨΨC
 ACGΨGAΨCCCCACCCΨGAAΨGGGGACGAΨCGGCACAAGAΨCGΨGAAΨGΨGGAΨCAGCGCCAGΨ
 ACGGCGAΨGΨGΨΨCAAGGGCGACCΨGAAΨCCCAAGCCCCAGGGCCAGCGGCΨGAΨCGAGGΨGA
 GCGΨGGAGGAGAAΨCACCCΨΨΨACCCΨGCGGGCCCCCAΨΨCAGAGGAΨCΨACGGGGΨGCGGΨ
 ACACCGAAACCΨGGAGCΨΨCCΨGCCAGCCΨGACCΨGΨACCGGCGACGCCGCCCGCCCAΨCCA
 GCACAΨCΨGCCΨGAAGCAΨACCACCΨGCΨΨCCAGGACGΨGGΨGGΨGGACGΨGGACΨGCGCCGA
 GAAΨACCAAGGAGGAΨCAGCΨGGCAGAGAΨCAGCΨACCGGΨΨΨCAGGGGAAGAAGGAGGCCGA
 CCAGCCΨΨGGAΨCGΨGGΨGAACACCAGCACCCΨGΨΨCGACGAGCΨGGAGCΨGGACCCΨCCCGAG
 AΨCGAGCCCCGGCΨGΨGAAGGΨGCΨGAGGACCGAGAAGCAGΨACCΨGGGGGΨGΨACAΨCΨGG
 AACAΨGCGGGGGAGCGAΨGGCACCAGCACAΨACGCCACCΨΨCCΨGGΨGACCΨGGAAGGGGGAC
 GAGAAGACΨCGGAACCCACCCΨGCΨGΨGACCCACAGCCCAGGGGCGCΨGAGΨΨCCACAΨGΨ
 GGAACΨACCAΨAGCCACGΨGΨΨCAGCGΨGGGGGACACCΨΨCΨCCCΨGGCΨAΨGCACCΨGCAGΨA

[illegible]

SEQ ID NO: 27 (MRNA 27)

[illegible]

SEQ ID NO: 28 (MRNA 28)

(G^{m7})ppp(A^{m2})UGGGGAYCCAAGCΨΨAGCΨΨGCΨΨGΨΨCΨΨΨΨΨGCAGAAGCΨCAGAAΨAAACGCΨCAACΨΨΨGGCGCCGCCACCAΨGGΨGCCCCAAGCCCΨGCΨGΨΨCGΨΨGCCCCΨGCΨGGGCΨΨCAG

SEQ ID NO: 29 (MRNA 29)

89

ΨGCCCCACGΨGΨACAAGCAGAΨCAGCGGCGΨGGACCCΨGGGCCΨGCCAACΨGGGGCAAGΨACGΨ
 GCΨGAΨGACCGCCGGCGCCAΨGAΨCGGCCΨGGΨGCΨGAΨCΨΨCAGCCΨGAΨGACCCΨGGΨGCAG
 AAGAGCCAACAGACCCGAGAGCAAGCAGCGGAGCΨΨCGGCCGACCCGGCAGAAACGΨGAGCGΨG
 ACAAGCCAAAGCGGCAAGGΨGAΨCCCΨAGCΨGGGAGAGCΨACAAGAGCGGCGGGCAGACAAGA
 CΨGΨGAΨAAGAAΨCΨΨAGGCΨGGAGCCΨCGGΨGGCCAΨGCΨΨCΨΨGCCCCΨΨGGGCCΨCCCCC
AGCCCCΨCCΨCCCCΨCCΨGCACCCGΨACCCCGΨGGΨCΨΨGAΨAAAGΨCΨGAGΨGGGCGGC
ACΨAGΨAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 30 (MRNA 30)

(G^{m7})ppp(A^{m2})ΨGGGGAΨCCAAGCΨΨAGCΨΨGCΨΨGΨΨCΨΨΨΨGCAGAAGCΨCAGAAΨAAACGC
 ΨCAACΨΨΨGGCGCCGCCACCAΨGGΨGCCCAAGCCΨGCΨGΨΨCGΨGCCCCΨGCΨGGGCΨΨCAG
 CCΨGΨGCΨΨCGGCAAGΨΨCCCCAΨCΨACACCAΨCCCCGACGAGCΨGGGCCCCΨGGAGCCCCAΨC
 GACAΨCCACCACCΨGAGCΨGCCCCAACAAACCΨGGΨGGΨCGAGGAΨGAAGGCΨGCACCAACCΨGA
 GCGAGΨΨCAGCΨACAΨGGAGCΨGAAGGΨGGGCΨACAΨCAGCGCCAΨCAAGGΨGAACGGCΨΨCA
 CCΨGCACCCGGCGΨGGΨGACCGAGGCCGAAACCΨACACCAACΨΨCGΨGGGCΨACGΨGACCAAC
 CΨΨCAAGAGAAAGCACΨΨCAGACCCACCCCGACGCCΨGCAGAGCCGCCΨACAACΨGGAAGAΨG
 GCCGGCGACCCΨAGAΨACGAGGAGAGCCΨGCAΨAACCΨΨACCCGACΨACCACΨGGCΨGAGAA
 CCGΨGAGAACCACCAAGGAGAGCCΨGAΨCAΨΨAΨCAGCCCΨAGCGΨGACCGACCΨGGACCCΨA
 CGACAAΨΨCCΨΨGCACAGCAGAGΨGΨΨCCCCGGCGGCAAGΨGCAGCGGCAΨCACCΨGAGCAGC
 ACCΨACΨGCAGCACCACCAACACGACΨACACCAΨCΨGGAΨGCCCCGAGAACCΨAGACCΨAGAACC
 CCΨGCGACACΨCΨACCAACAGCAGAGGCAAGAGAGCΨAGCAAGGGCAACAAGACCΨGCGGCΨΨ
 CGΨGGACGAGAGAGGCCΨGΨACAAGAGCCΨGAAGGGCGCCΨGCAGACΨGAAGCΨGΨGCGGCΨ
 GCΨGGGCCΨGAGACΨGAΨGGACGGCACCCΨGGGΨGGCCAΨGCAGACAAGCGACGAAACCAAGΨG
 GΨGCCCCCCCCGAΨCAGCΨGGΨGAACCΨGCACGACΨΨCAGAAGCGACGAGAΨCGAGCACCΨGGΨG
 GΨGGAGGAGCΨGGΨGAAGAAGAGAGAGGAGΨGCCΨGGACGCCΨGGAGAGCAΨCAΨGACCACC
 AAGAGCGΨGΨCCΨΨCAGAAGACΨGAGCCACCΨGAGAAAGCΨGGΨGCCCGGCΨΨCGGCAAGGCC
 ΨACACCAΨCΨΨCAACAAGACCCΨGAΨGGAGGCCGACGCCACΨACAAAAGCGΨGAGAACCΨGGA
 ACGAGAΨCAΨCCΨΨAGCAAGGGCΨGCCΨGAAGGΨGGGCGGCAGAΨGCCACCCCCAΨGΨGAACGG
 CGΨGΨΨCΨΨCAACGGCAΨCAΨCCΨGGGCCCGACGGCCACGΨGCΨGAΨCCCCGAGAΨGCAGAGC
 AGCCΨGCΨGCAGCAGCACAΨGGAGCΨGCΨGAAGAGCAGCGΨGAΨCCCCΨGAΨGCACCCCCΨGG
 CCGACCCΨAGCACCΨGΨΨCAAGGAGGGCGACGAGGCCGAGGACΨΨCGΨGGAGGΨGCACCΨGCC
 CGACGΨGΨACAAGCAGAΨCAGCGGCGΨGGACCΨGGGCCΨGCCAACΨGGGGCAAGΨACGΨGCΨ
 GAΨGACCGCCGGCGCCAΨGAΨCGGCCΨGGΨGCΨGAΨCΨΨCAGCCΨGAΨGACCΨGGΨGCAGAAG
 AGCCAACAGACCCGAGAGCAAGCAGCGGAGCΨΨCGCGGCACCCGGCAGAAACGΨGAGCGΨGACA
 AGCCAAAGCGGCAAGGΨGAΨCCΨΨAGCΨGGGAGAGCΨACAAGAGCGGCGGGCAGACAAGACΨG
ΨGAΨAAGAAΨCΨΨAGGCΨGGAGCCΨCGGΨGGCCAΨGCΨΨCΨΨGCCCCΨΨGGGCCΨCCCCCAGC
CCCΨCCΨCCCCΨCCΨGCACCCGΨACCCCGΨGGΨCΨΨGAΨAAAGΨCΨGAGΨGGGCGGCACΨ
AGΨAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 31 (MRNA 31)

(G^{m7})ppp(A^{m2})UGGGGAΨCCAAGCΨΨAGCΨΨGCΨΨGΨΨCΨΨΨΨGCAGAAGCΨCAGAAΨAAACGC
 ΨCAACΨΨΨGGCGCCGCCACCAΨGGΨGAGCAAGGGCGAGGAGCΨGΨΨCACCGGGGΨGGΨGCCCA
 ΨCCΨGGΨCGAGCΨGGACGGCGACGΨAAACGGCCACAAGΨΨCAGCGΨGΨCCGGCGAGGGCGAGG
 GCGAΨGCCACCΨACGGCAAGCΨGACCCΨGAAGΨΨCAΨCΨGCACCACCCGGCAAGCΨGCCCGΨGCC
 CΨGGCCCACCCΨCGΨGACCACCCΨGACCΨACGGCGΨGCAGΨGCΨΨCAGCCGCΨACCCGACCAC
 AΨGAAGCAGCAGACΨCΨΨCAAGΨCCGCCAΨGCCCGAAGGCΨACGΨCCAGGAGCGCACCAΨCΨ
 ΨCΨΨCAAGGACGACGGCAACΨACAAGACCCGCGCCGAGGΨGAAGΨΨCGAGGGCGACACCCΨGGΨ
 GAACCGCAΨCGAGCΨGAAGGGCAΨCGACΨΨCAAGGAGGACGGCAACAΨCCΨGGGGCACAAGCΨ
 GGAGΨACAACΨACAACAGCCACAACGΨCΨAΨAΨCAΨGGCCGACAAGCAGAAGAAGCGCAΨCAA

GGΨGAACΨΨCAAGAΨCCGCCACAACAΨCGAGGACGGCAGCGΨGCAGCΨCGCCGACCACΨACCAG
CAGAACACCCCCAΨCGGCGACGGCCCCGΨGCΨGCΨGCCCGACAACCACΨACCΨGAGCACCCAGΨ
CCGCCCΨGAGCAAAGACCCCAACGAGAAGCGCGAΨCACAΨGGΨCCΨGCΨGGAGΨΨCGΨGACCGC
CGCCGGGAΨCACΨCΨCGGCAΨGGACGAGCΨGΨACAAGΨGAΨAAGAAΨΨCΨAGGCΨGGAGCCΨC
GGΨGGCCAΨGCGΨΨCΨΨGCCCCΨΨGGGCCΨCCCCCAGCCCCΨCCΨCCCCΨCCΨGCACCCGΨACC
CCCGΨGGΨCΨΨΨGAAΨAAAGΨCΨGAGΨGGGCGGCACΨAGΨAAAAAAAAAAAAAAAAAAAAAAAAA
AAA

SEQ ID NO: 32 (MRNA 32)

(G^{m7,3'OMe})ppp(A^{m2})UGGGGAΨCCAAGCΨΨAGCΨΨGCΨΨGΨΨCΨΨΨΨGCAGAAGCΨCAGAAΨAAA
CGCΨCAACΨΨΨGGCGCCGCCACCAΨGGΨGAGCAAGGGCGAGGAGCΨGΨΨCACCAGGGGΨGGΨGCC
CAΨCCΨGGΨCGAGCΨGGACGGCGACGΨAAACGGCCACAAGΨΨCAGCGΨGΨCCGGCGAGGGCGA
GGGCGAΨGCCACCΨACGGCAAGCΨGACCCΨGAAGΨΨCAΨCΨGCACCACCGGCAAGCΨGCCCGΨG
CCCΨGGCCCCACCCΨCGΨGACCACCCΨGACCΨACGGCGΨGCAGΨGCΨΨCAGCCGCΨACCCCGACC
ACAΨGAAGCAGCACGACΨΨCΨΨCAAGΨCCGCCAΨGCCCCGAAGGCΨACGΨCCAGGAGCGCACCAΨC
CΨΨCΨΨCAAGGACGACGGCAACΨACAAGACCCGCGCCGAGGΨGAAGΨΨCGAGGGCGACACCCΨG
GΨGAACCGCAΨCGAGCΨGAAGGGCAΨCGACΨΨCAAGGAGGACGGCAACAΨCCΨGGGGCACAAG
CΨGGAGΨACAACΨACAACAGCCACAACGΨCΨAΨAΨCAΨGGCCGACAAGCAGAAGAACGGCAΨC
AAGGΨGAACΨΨCAAGAΨCCGCCACAACAΨCGAGGACGGCAGCGΨGCAGCΨCGCCGACCACΨACC
AGCAGAACACCCCCAΨCGGCGACGGCCCCGΨGCΨGCΨGCCCGACAACCACΨACCΨGAGCACCCA
GΨCCGCCΨGAGCAAAAGACCCCAACGAGAAGCGCGAΨCACAΨGGΨCCΨGCΨGGAGΨΨCGΨGACC
GCCGCCGGGAΨCACΨCΨCGGCAΨGGACGAGCΨGΨACAAGΨGAΨAAGAAΨΨCΨAGGCΨGGAGCC
ΨCGGΨGGCCAΨGCGΨΨCΨΨGCCCCΨΨGGGCCΨCCCCCAGCCCCΨCCΨCCCCΨCCΨGCACCCGΨ
ACCCCGΨGGΨCΨΨΨGAAΨAAAGΨCΨGAGΨGGGCGGCACΨAGΨAAAAAAAAAAAAAAAAAAAAAAAAA
AAA
AA

SEQ ID NO: 33 (MRNA 33)

(G^{m7})ppp(A^{m2})ΨGGGGAΨCCAAGCΨΨAGCΨΨGCΨΨGΨΨCΨΨΨΨGCAGAAGCΨCAGAAΨAAACGC
ΨCAACΨΨΨGGCGCCGCCACCAΨGGΨGAGCAAGGGCGAGGAGCΨGΨΨCACCAGGGGΨGGΨGCCCA
ΨCCΨGGΨCGAGCΨGGACGGCGACGΨAAACGGCCACAAGΨΨCAGCGΨGΨCCGGCGAGGGCGAGG
GCGAΨGCCACCΨACGGCAAGCΨGACCCΨGAAGΨΨCAΨCΨGCACCACCGGCAAGCΨGCCCGΨGCC
CΨGGCCCCACCCΨCGΨGACCACCCΨGACCΨACGGCGΨGCAGΨGCΨΨCAGCCGCΨACCCCGACCAC
AΨGAAGCAGCACGACΨΨCΨΨCAAGΨCCGCCAΨGCCCCGAAGGCΨACGΨCCAGGAGCGCACCAΨCΨ
ΨCΨΨCAAGGACGACGGCAACΨACAAGACCCGCGCCGAGGΨGAAGΨΨCGAGGGCGACACCCΨGGΨ
GAACCGCAΨCGAGCΨGAAGGGCAΨCGACΨΨCAAGGAGGACGGCAACAΨCCΨGGGGCACAAGCΨ
GGAGΨACAACΨACAACAGCCACAACGΨCΨAΨAΨCAΨGGCCGACAAGCAGAAGAACGGCAΨCAA
GGΨGAACΨΨCAAGAΨCCGCCACAACAΨCGAGGACGGCAGCGΨGCAGCΨCGCCGACCACΨACCAG
CAGAACACCCCCAΨCGGCGACGGCCCCGΨGCΨGCΨGCCCGACAACCACΨACCΨGAGCACCCAGΨ
CCGCCCΨGAGCAAAGACCCCAACGAGAAGCGCGAΨCACAΨGGΨCCΨGCΨGGAGΨΨCGΨGACCGC
CGCCGGGAΨCACΨCΨCGGCAΨGGACGAGCΨGΨACAAGΨGAΨAAGAAΨΨCΨAGGCΨGGAGCCΨC
GGΨGGCCAΨGCGΨΨCΨΨGCCCCΨΨGGGCCΨCCCCCAGCCCCΨCCΨCCCCΨCCΨGCACCCGΨACC
CCCGΨGGΨCΨΨΨGAAΨAAAGΨCΨGAGΨGGGCGGCACΨAGΨAAAAAAAAAAAAAAAAAAAAAAAAA
AAA

SEQ ID NO: 34 (MRNA 34)

(G^{m7})vppp(A^{m2})UGGGGAΨCCAAGCΨΨAGCΨΨGCΨΨGΨΨCΨΨΨΨGCAGAAGCΨCAGAAΨAAACG
CΨCAACΨΨΨGGCGCCGCCACCAΨGGΨGAGCAAGGGCGAGGAGCΨGΨΨCACCAGGGGΨGGΨGCCCA
ΨCCΨGGΨCGAGCΨGGACGGCGACGΨAAACGGCCACAAGΨΨCAGCGΨGΨCCGGCGAGGGCGAGG
GCGAΨGCCACCΨACGGCAAGCΨGACCCΨGAAGΨΨCAΨCΨGCACCACCGGCAAGCΨGCCCGΨGCC

CΨGGCCACCCΨCGΨGACCACCCΨGACCΨACGGCGΨGCAGΨGCΨΨCAGCCGCΨACCCCGACCAC
AΨGAAGCAGCAGACΨΨCΨΨCAAGΨCCGCCAΨGCCCGAAGGCΨACGΨCCAGGAGCGCACCAΨCΨ
ΨCΨΨCAAGGACGACGGCAACΨACAAGACCCGCGCCGAGGΨGAAGΨΨCGAGGGCGACACCCΨGGΨ
GAACCGCAΨCGAGCΨGAAGGGCAΨCGACΨΨCAAGGAGGACGGCAACAΨCCΨGGGGCACAAGCΨ
GGAGΨACAACΨACAACAGCCACAACGΨCΨAΨAΨCAΨGGCCGACAAGCAGAAGAACGGCAΨCAA
GGΨGAACΨΨCAAGAΨCCGCCACAACAΨCGAGGACGGCAGCGΨGCAGCΨCGCCGACCACΨACCAG
CAGAACACCCCCAΨCGGCGACGGCCCCGΨGCΨGCΨGCCCGACAACCACΨACCΨGAGCACCCAGΨ
CCGCCΨGAGCAAAGACCCCAACGAGAAGCGCGAΨCACAΨGGΨCCΨGCΨGGAGΨΨCGΨGACCGC
CGCCGGGAΨCACΨCΨCGGCAΨGGACGAGCΨGΨACAAGΨGAΨAAGAAΨΨCΨAGGCΨGGAGCCΨC
GGΨGGCCAΨGCΨΨCΨΨGCCCCΨΨGGGCCΨCCCCCAGCCCCΨCCΨCCCCΨCCΨGCACCCGΨACC
CCCGΨGGΨCΨΨGAAΨAAAGΨCΨGAGΨGGGCGGCACΨAGΨAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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CLAIMS

1. A modified RNA molecule, wherein the modified RNA molecule comprises a 5' cap, wherein the RNA molecule is not self-replicable, wherein the 5' end of the modified RNA molecule has the sequence of N1pppN2pU or N1vpppN2pU, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine, and wherein at least one uridine in the molecule is a modified uridine except for the first 5' uridine.
2. The modified RNA molecule of claim 1, wherein the 5' end of the modified RNA molecule has the sequence of GpppApU or GvpppApU, wherein A is modified or unmodified.
3. The modified RNA molecule of claim 1 or 2, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GpppApU or m⁷GvpppApU.
4. The modified RNA molecule of claim 2 or 3, wherein the A is modified, preferably 2'-O-methylated.
5. The modified RNA molecule of any one of claims 1-4, wherein the 5' end of the modified RNA molecule has the sequence of m⁷GpppA_mpU or m⁷GvpppA_mpU.
6. The modified RNA molecule of any one of claims 1-5, wherein all of the uridines in the molecule are modified except for the first 5' uridine,
preferably, all of the uridines in the molecule are m¹Ψ except for the first 5' uridine.
7. The modified RNA molecule of any one of claims 1-6, comprising an open reading frame comprising a coding sequence,
preferably, the coding sequence encodes eGFP, SARS-COV-2 BA.4/BA.5 RBD, a rabies virus antigen, a respiratory syncytial virus antigen or a varicella-zoster virus antigen.
8. The modified RNA molecule of any one of claims 1-7, wherein the molecule further comprises at least one modified G, C, or A.
9. The modified RNA molecule of any one of claims 1-8, wherein the molecule has a modified backbone,
preferably, the modified backbone comprising at least one phosphorothioate linkage.

10. A pharmaceutical composition comprising the modified RNA of any one of claims 1-9, further comprising a pharmaceutically acceptable carrier.
11. The pharmaceutical composition of claim 10, wherein the pharmaceutically acceptable carrier is an LNP,
preferably, the LNP comprises a cationic lipid,
more preferably, the LNP comprises: i) between about 30 to 55 mol percent of a cationic lipid; ii) between about 5 to 40 mol percent of a phospholipid; iii) between about 20 to 50 mol percent of a sterol; and iv) a polymer conjugated lipid.
12. A method of *in vitro* transcribing a DNA molecule to a modified RNA molecule, comprising combining the DNA molecule with an *in vitro* transcription mix comprising a DNA-dependent RNA polymerase, modified and/or unmodified nucleotides, and a 5' cap, wherein the modified RNA molecule is non-self-replicable, wherein the 5' cap has the sequence of N1pppN2pU or N1vpppN2pU, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine, wherein the *in vitro* transcription mix comprises modified uridines, and wherein at least one uridine in the modified RNA molecule is a modified uridine except for the first 5' uridine.
13. The method of claim 12, wherein the 5' cap has the sequence of GpppApU or GvpppApU, wherein A is modified or unmodified.
14. The method of claim 12 or 13, wherein the 5' cap has the sequence of m⁷GpppApU or m⁷GvpppApU.
15. The method of claim 13 or 14, wherein the A is modified, preferably 2'-O-methylated.
16. The method of any one of claims 12-15, wherein the 5' cap has the sequence of m⁷GpppA_mpU or m⁷GvpppA_mpU.
17. The method of any one of claims 12-16, wherein all of the uridines in the modified RNA molecule are modified except for the first 5' uridine.
18. The method of any one of claims 12-17, wherein the modified uridines are m¹Ψ,

preferably, all of the uridines in the modified RNA molecule are m¹Ψ except for the first 5' uridine.

19. The method of any one of claims 12-18, wherein the DNA-dependent RNA polymerase is a T7 RNA polymerase or the variant thereof.

20. The method of any one of claims 12-19, further comprising isolating the modified RNA molecule.

21. A modified RNA molecule produced by the method of any one of claims 12-20.

22. A kit comprising: 1) a DNA-dependent RNA polymerase; 2) modified and/or unmodified nucleotides, and 3) a 5' cap, and 4) an instruction for carrying out the method of any one of claims 12-20, wherein the 5' cap has the sequence of N1pppN2pU or N1vpppN2pU, wherein U is an unmodified uridine, and wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine.

23. A method of lowering the innate immune stimulation of a RNA molecule, comprising using a 5' cap having the sequence of N1pppN2pU or N1vpppN2pU and modifying at least one uridine in the RNA molecule except for the first 5' uridine to produce the RNA molecule, wherein the RNA molecule is not self-replicable, wherein U is an unmodified uridine, wherein each of N1 and N2 is independently a modified or unmodified nucleotide other than a modified or unmodified uridine,

preferably, the 5' cap has the sequence of GpppApU or GvpppApU, wherein A is modified or unmodified,

preferably, the 5' cap has the sequence of m⁷GpppApU or m⁷GvpppApU,

preferably, the 5' cap has the sequence of m⁷GpppA_mpU or m⁷GvpppA_mpU,

preferably, the method comprises modifying all of the uridines in the RNA molecule except for the first 5' uridine,

more preferably, the method comprises modifying all of the uridines in the RNA molecule to m¹Ψ except for the first 5' uridine.