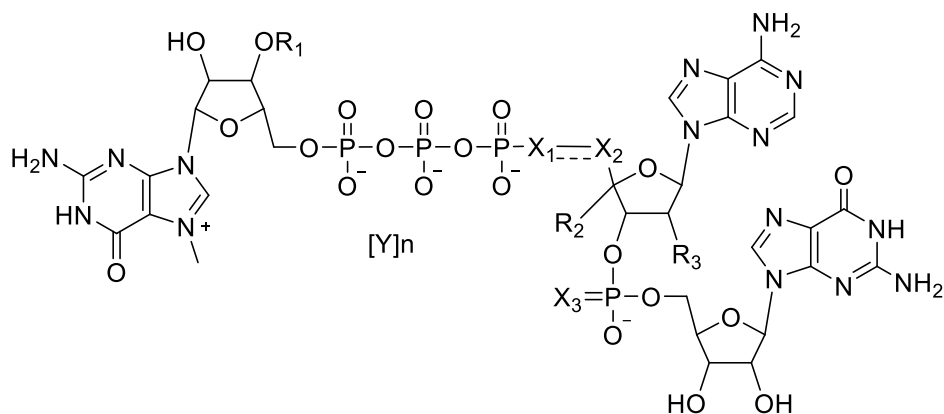
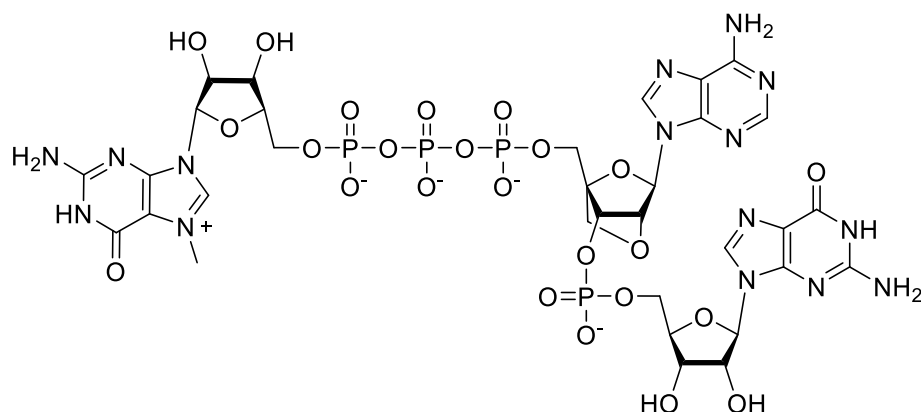


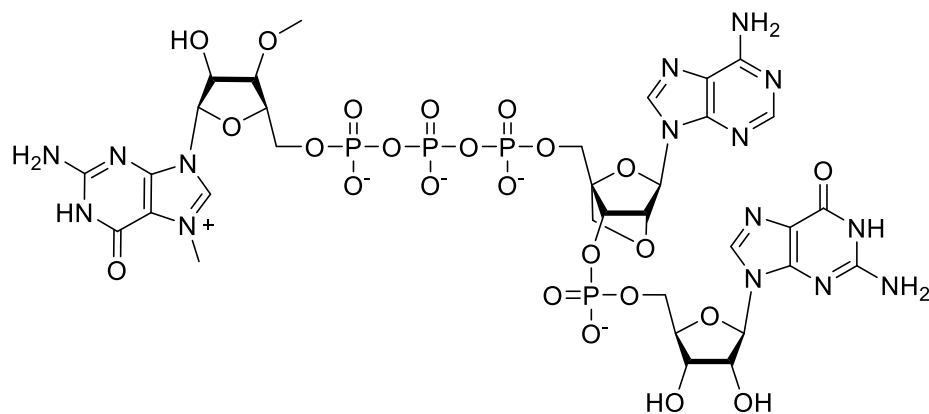


Formula I

or any stereoisomer thereof, wherein: $\equiv$ is a single bond or a double bond; R_1 is H or CH_3 ; R_2 is H and R_3 is OCH_3 or F, or R_2 and R_3 are covalently bonded together and, together with intermediate atoms, form a 2'-O, 4'-C methylene bridge; X_1 is O or CH; X_2 is CH_2 or CH; X_3 is O or S; each independent Y is H^+ or a cation; and n is 0, 1, 2, 3, or 4, wherein when X_1 is CH, X_2 is CH and $\equiv$ is a double bond. Optionally, the compound has the following structure:



Compound 1

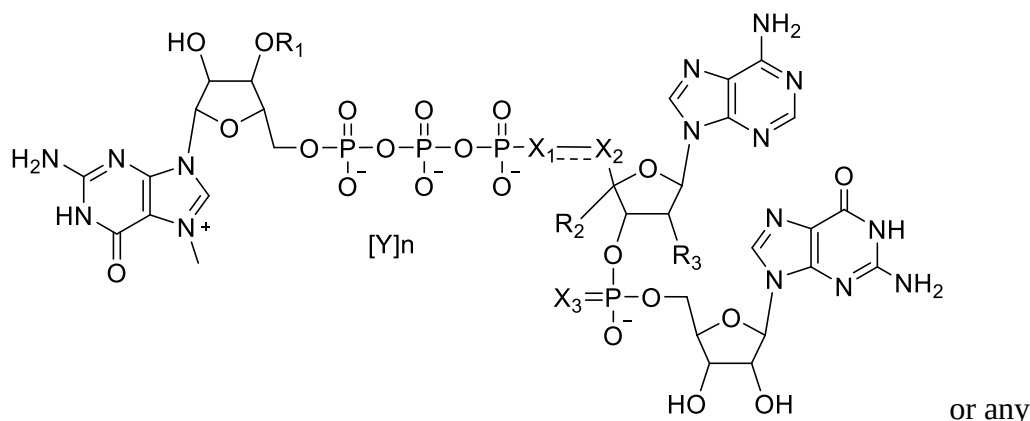


Compound 2

specific order, it is no way intended that an order be inferred, in any respect. This holds for any possible non-express basis for interpretation, including: matters of logic with respect to arrangement of steps or operational flow; plain meaning derived from grammatical organization or punctuation; and the number or type of embodiments described in the specification.

II. Compounds

A class of compounds described herein is represented by **Formula I**:



stereoisomer thereof.

In **Formula I**, $\equiv$ is a single bond or a double bond.

Also in **Formula I**, R_1 is H or CH_3 .

Additionally in **Formula I**, R_2 is H and R_3 is OCH_3 or F, or R_2 and R_3 are covalently bonded together and, together with intermediate atoms, form a 2'-O, 4'-C methylene bridge.

Further in **Formula I**, X_1 is O or CH.

Also in **Formula I**, X_2 is CH_2 or CH.

Additionally in **Formula I**, X_3 is O or S.

Further in **Formula I**, each independent Y is H^+ or a cation and n is 0, 1, 2, 3, or 4.

Optionally, the cation is a pharmaceutically acceptable cation (e.g., Na^+ , K^+ , Li^+ , or $TEAH^+$).

In **Formula I**, when X_1 is CH, then X_2 is CH and $\equiv$ is a double bond.

As understood by those of skill in the art, the structures shown of the compounds described herein are representations of one form of the compound. Although such compounds may be drawn or described in protonated (free acid) form, in ionized (anionic) form, or ionized and in association with one or more cations (salt form), aqueous solutions of such compounds exist in equilibrium among such forms. For example, a phosphate linkage of a compound described herein, in aqueous solution, exists in equilibrium among free acid, anion, and salt forms.

below:



wherein

5 $\dashv$, R_1 , R_2 , R_3 , X_1 , X_2 , and X_3 are as defined above for **Formula I**.

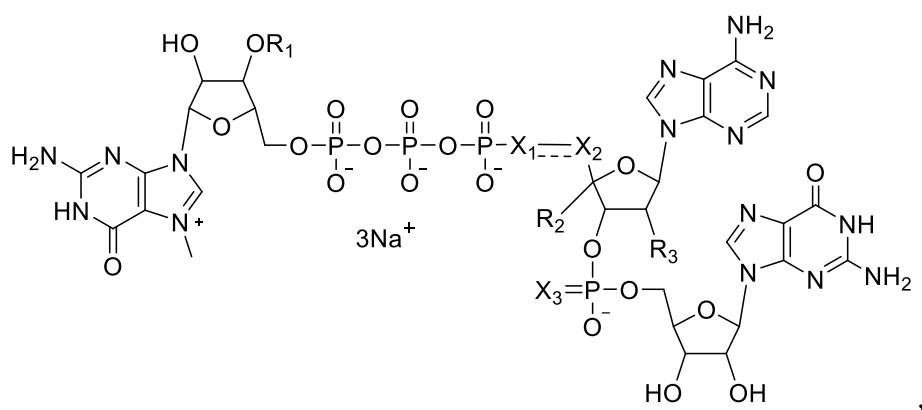
Optionally, **Formula I** can be depicted in ionized (anionic) form as shown below:



wherein

$\equiv$, R_1 , R_2 , R_3 , X_1 , X_2 , and X_3 are as defined above for **Formula I**.

Optionally, **Formula I** can be depicted in ionized and in association with one or more cations (salt) form as shown below, in which compounds of the structure are in association with sodium cations (for representation purposes only and not by way of limitation):

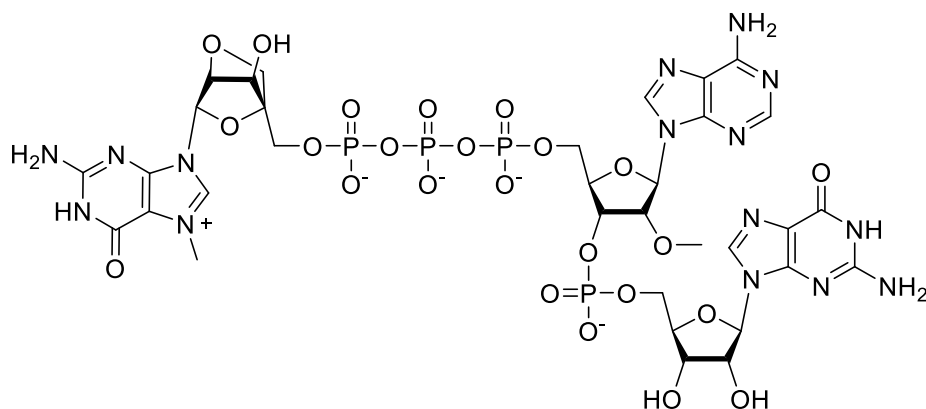


wherein

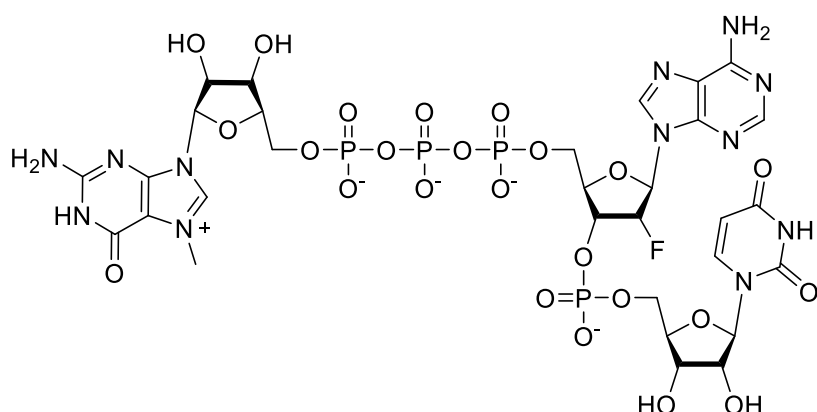
==, R₁, R₂, R₃, X₁, X₂, and X₃ are as defined above for **Formula I**. As understood by those of skill in the art, the cation salt form may exist in which one, two, three, or four cations are present.

Unless otherwise indicated, compounds described herein are intended to include all such forms. Moreover, certain compounds have several such linkages, each of which is in equilibrium. Thus, compounds in solution exist in an ensemble of forms at multiple positions all at equilibrium. Drawn structures necessarily depict a single form. Nevertheless, unless otherwise indicated, such drawings are likewise intended to include corresponding forms. Herein, a structure depicting the free acid of a compound followed by the term “or salts thereof” expressly includes all such forms that may be fully or partially protonated/deprotonated/in association with a cation. In certain instances, one or more specific cation is identified. In certain methods, compounds disclosed herein are in aqueous solution with sodium. In certain methods, compounds are in aqueous solution with potassium. In certain methods, compounds are in aqueous solution with lithium. In certain methods, compounds are in aqueous solution with triethylammonium.

As described above, **Formula I** can include compounds in which R₂ and R₃ are covalently bonded together and, together with intermediate atoms, form a 2'-O, 4'-C methylene bridge. The 2'-O, 4'-C methylene bridge moiety can also be referred to herein as a linked nucleic acid (LNA) moiety. Optionally, the compounds of **Formula I** having an LNA moiety are represented by **Structure I-A** as shown below, or a salt thereof:



Compound 32



Compound 33

The mRNA dose optionally comprises less than 7 ng of double stranded RNA (dsRNA) per 1 µg of mRNA, wherein the subject exhibits increased tolerability to the administered dose of the mRNA as compared to an equivalent dose of the mRNA comprising 7 ng/µg or greater dsRNA. Optionally, the increased tolerability is determined by measuring one or more of

5 body weight, organ weight, aspartate aminotransferase (AST) levels, alanine transaminase (ALT) levels, C-reactive protein (CRP) levels, procalcitonin (PCT) levels, interleukin-6 (IL-6) levels, erythrocyte sedimentation rate (ESR), serum amyloid A levels, and serum ferritin levels prior to the administering and a period of time after the administering.

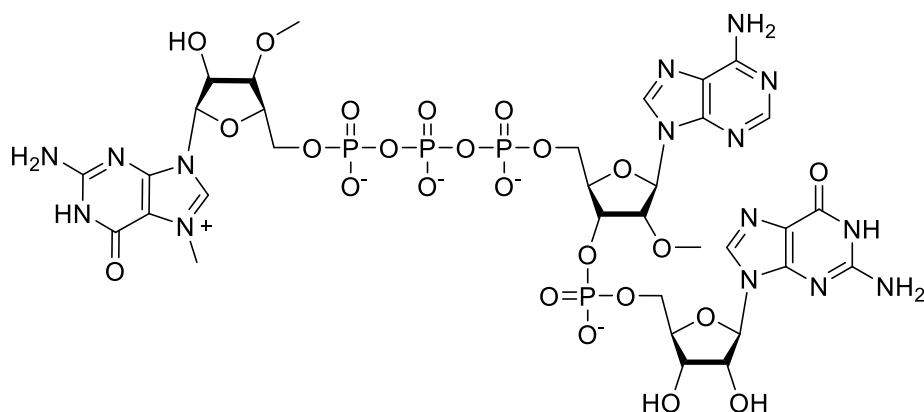
In some cases, the increased tolerability is measured by testing in a standard *in vivo* assay. As described above, the term “*in vivo* assays” refer to methods used to detect and/or

10 measure capacity of one or more of the compounds or molecules including the compounds (e.g., mRNA molecules in, for example, a therapeutic dose) to increase or decrease a property relative to a control (e.g., biomarker levels). Optionally, *in vivo* assays as described herein can be used to determine a subject’s tolerability levels to a given compound or molecule.

15 Exemplary measurements for assessing tolerability include one or more of body weight, organ weight, aspartate aminotransferase (AST) levels, alanine transaminase (ALT) levels, C-reactive protein (CRP) levels, procalcitonin (PCT) levels, interleukin-6 (IL-6) levels, erythrocyte sedimentation rate (ESR), serum amyloid A levels, and serum ferritin levels.

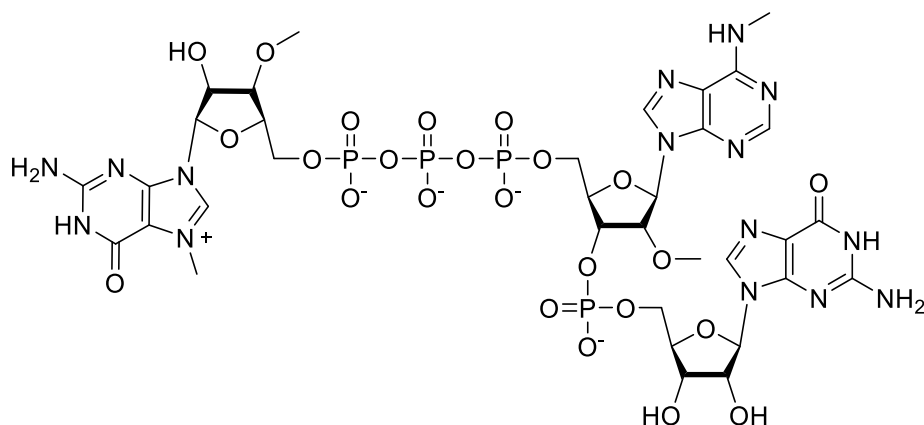
In some examples, the methods include administering to the subject a therapeutic dose unit comprising an effective amount of an mRNA encoding a polypeptide, wherein the mRNA comprises a 5'-cap having the following formula ${}^m7G_3'\text{OMe}pppA^1_2'\text{OMe}G$ and wherein A^1 is adenosine, N6-methyladenosine, diaminopurine. A^1 can optionally include an LNA moiety. The ${}^m7G_3'\text{OMe}pppA^1_2'\text{OMe}G$ compound for use in the methods for increasing translation of a polypeptide in a subject can include one or more of the following compounds:

${}^m7G_3'\text{OMe}pppA_2'\text{OMe}pG$:



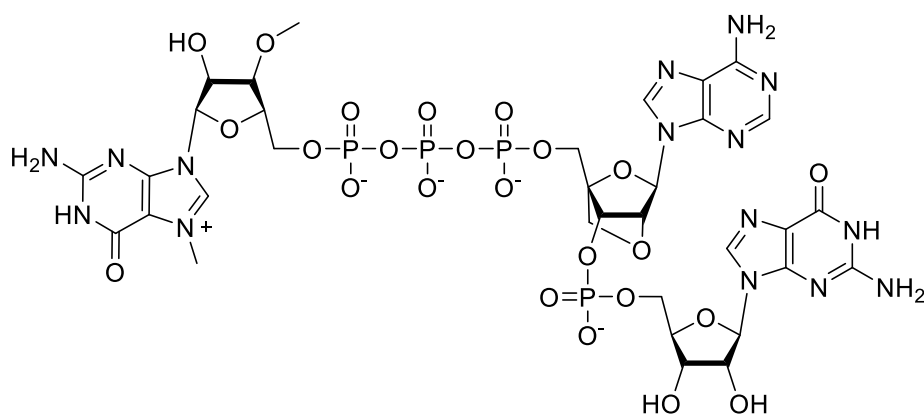
Compound 29

${}^m7G_3'\text{OMe}ppp{}^m6A_2'\text{OMe}pG$:



Compound 30

${}^m7G_3'\text{OMe}pppA_2',4'\text{-LNA}pG$:

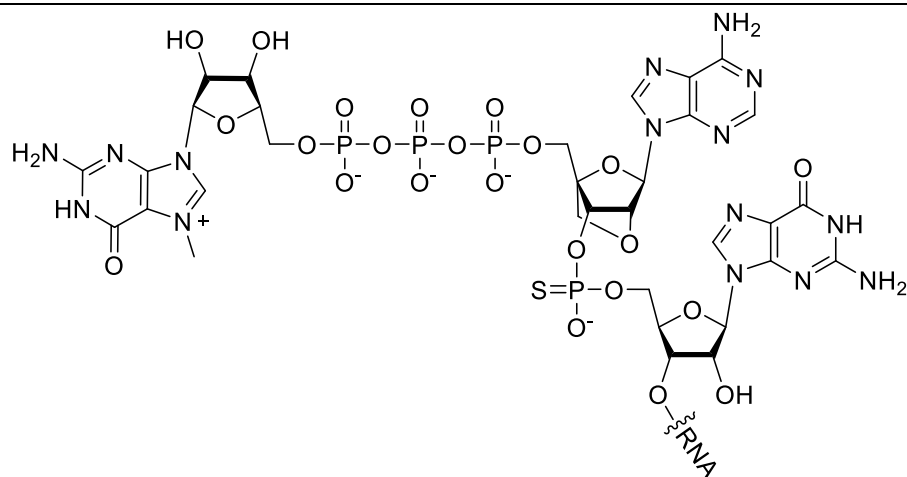


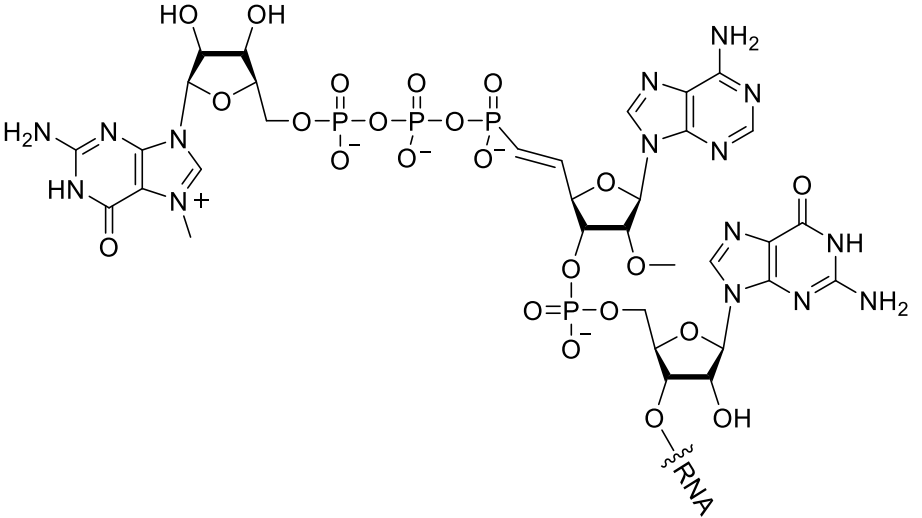
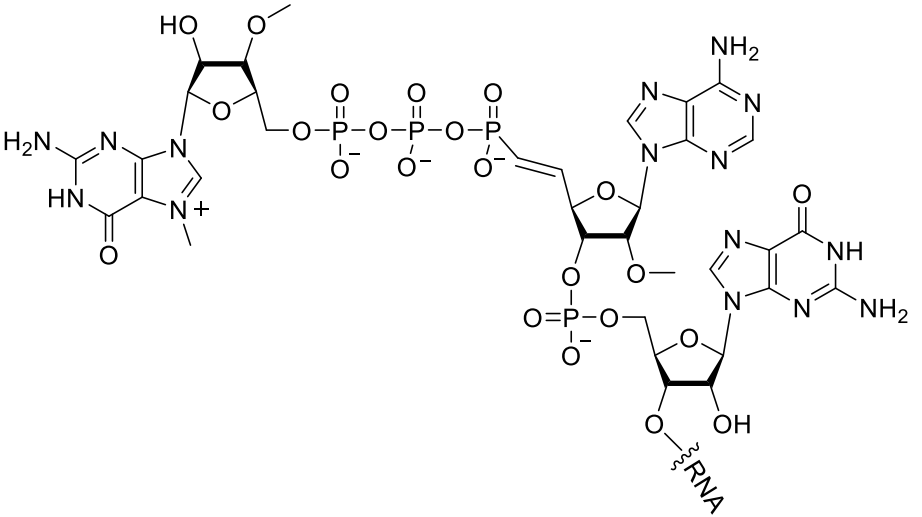
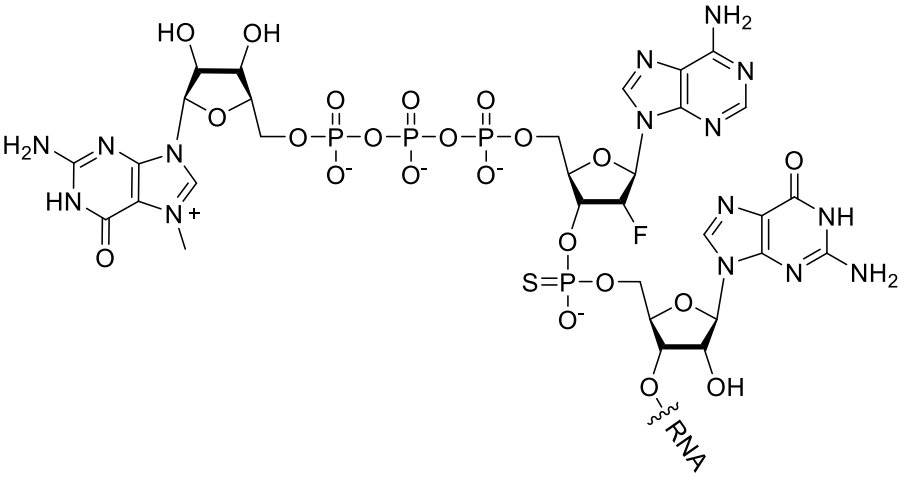
Compound 2

Table 1. Synthesized mRNAs

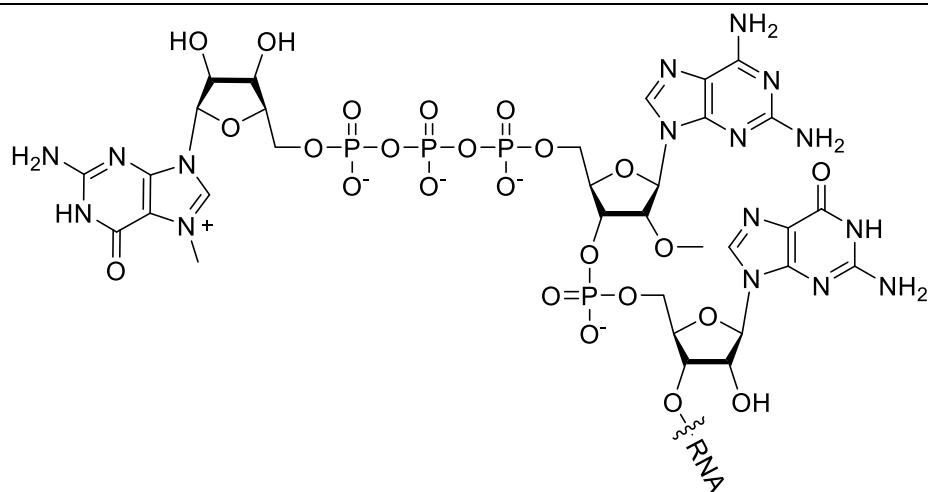
Cap Analog	Synthesized mRNA Structure
$m^7GpppA_{2'}OMe pG$ (Control)	
$m^7GpppA_{2',4'}-LNAPG$ (Compound 1)	
$m^7G_{3'}OMe pppA_{2',4'}-LNAPG$ (Compound 2)	

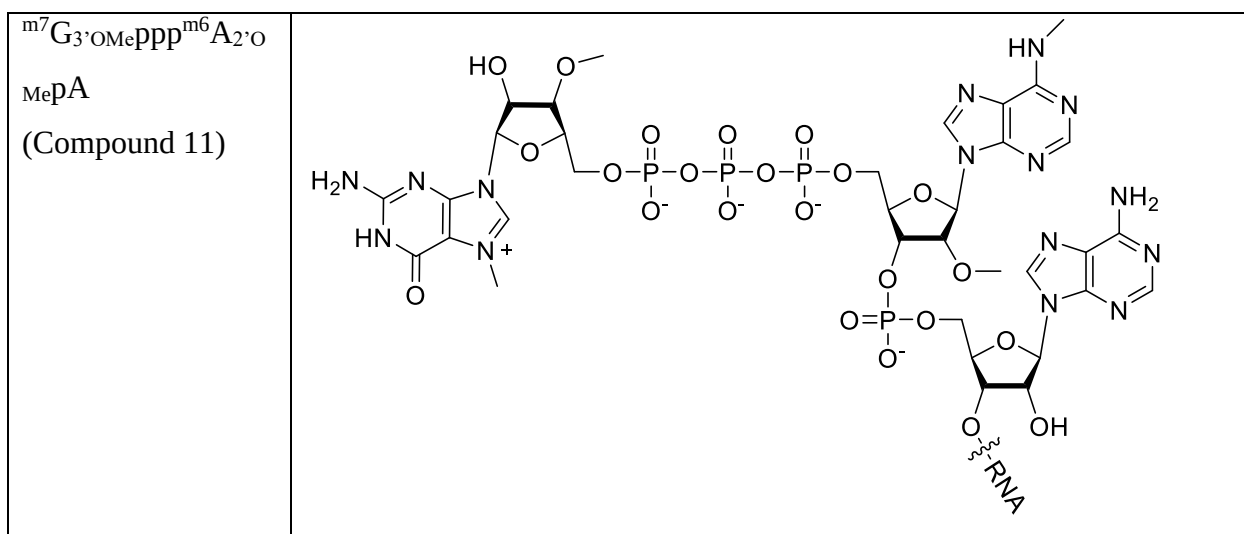
^{m7}GpppA_{2'-4'}-LNAP(s)G
(Compound 3)

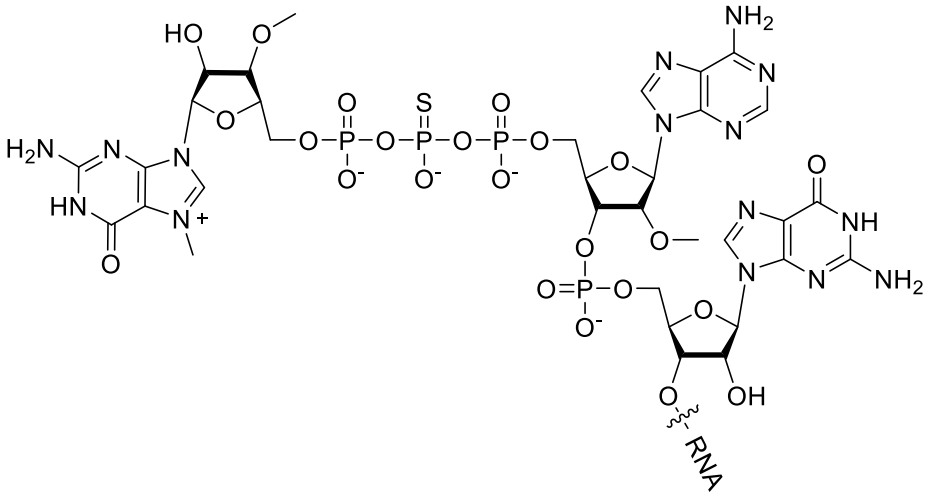
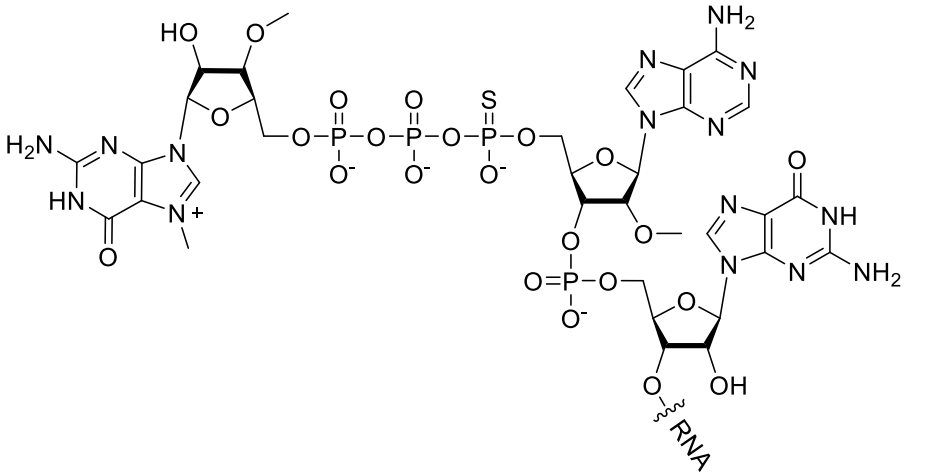
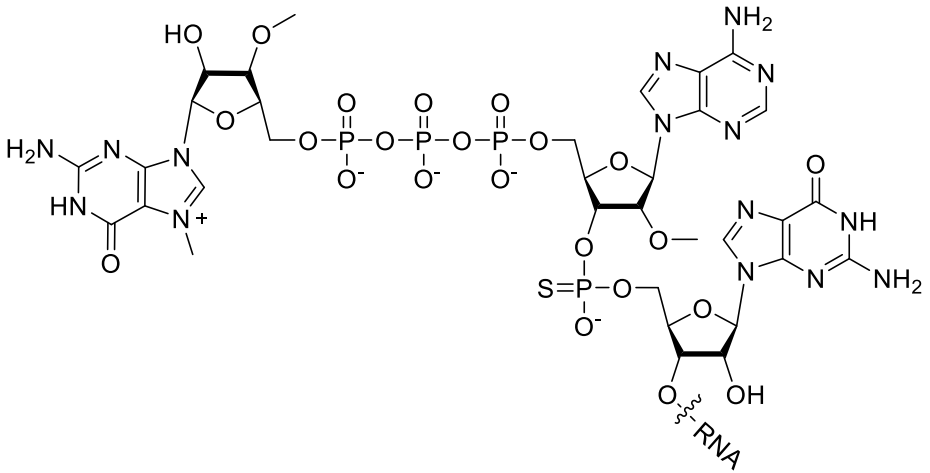


$m^7GpppA_2'OMe-$ 5'-vinylpG (Compound 4)	
$m^7G_{3'}-OMepppA_2'OMe-$ 5'-vinylpG (Compound 5)	
$m^7GpppA_2'FP(s)G$ (Compound 6)	

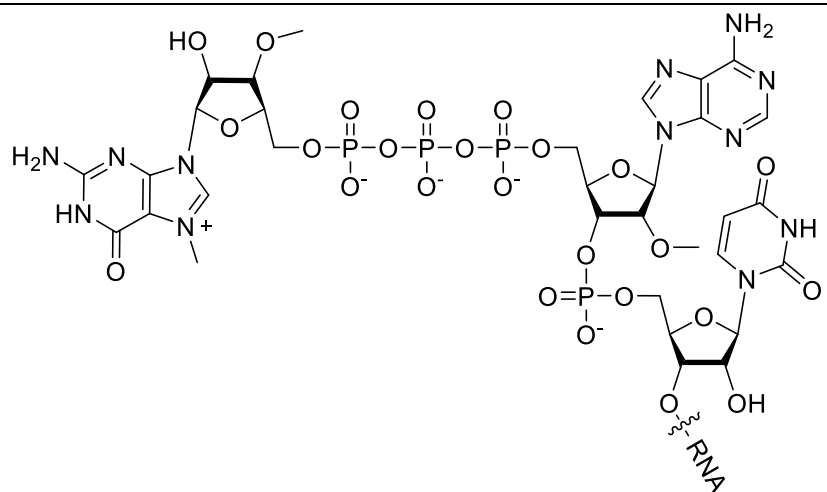
^{m7}Gppp(diamino-
purine)₂'OMe pG
(Compound 7)





$m^7G_{3'}^{OMe}pp(s)pA_{2'}^{OMe}$ pG* (Compound 19) *Two diastereomers separated: $m^7G_{3'}^{OMe}pp(s_{Rp})pA_{2'}^{OMe}$ ePG and $m^7G_{3'}^{OMe}pp(s_{Sp})pA_{2'}^{OMe}$ ePG	
Synthesis of $m^7G_{3'}^{OMe}ppp(s)A_{2'}^{OMe}$ pG (Compound 20)	
$m^7G_{3'}^{OMe}pppA_{2'}^{OMe}p(s)$)G* (Compound 21) *Two diastereomers separated: $m^7G_{3'}^{OMe}pppA_{2'}^{OMe}p(s_{Rp})G$ and $m^7G_{3'}^{OMe}pppA_{2'}^{OMe}p(s_{Sp})G$	

^{m7}G_{3'}OMepppA_{2'}OMepU
(Compound 22)



$\text{m}^7\text{G}_2'\text{OH},$
 $2'\text{MepppA}_2'\text{OMePG}$
 (Compound 43)

