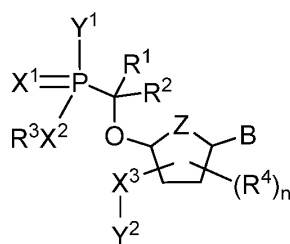


CLAIMS

We claim:

1. A nucleic acid or analogue thereof comprising a 4'-O-methylene phosphonate internucleotide linkage, wherein the 4'-O-methylene phosphonate internucleotide linkage is represented by formula I:



I

or a pharmaceutically acceptable salt thereof, wherein:

B is a nucleobase or hydrogen;

R¹ and R² are independently hydrogen, halogen, R⁵, -CN, -S(O)R, -S(O)₂R, -Si(OR)₂R, -Si(OR)R₂, or -SiR₃, or:

R¹ and R² on the same carbon are taken together with their intervening atoms to form a 3-7 membered saturated or partially unsaturated ring having 0-3 heteroatoms, independently selected from nitrogen, oxygen, and sulfur;

each R is independently hydrogen, a suitable protecting group, or an optionally substituted group selected from C₁-₆ aliphatic, phenyl, a 4-7 membered saturated or partially unsaturated heterocyclic having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and a 5-6 membered heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or:

two R groups on the same atom are taken together with their intervening atoms to form a 4-7 membered saturated, partially unsaturated, or heteroaryl ring having 0-3 heteroatoms, independently selected from nitrogen, oxygen, silicon, and sulfur;

R³ is hydrogen, a suitable protecting group, a suitable prodrug, or an optionally substituted group selected from C₁-₆ aliphatic, phenyl, a 4-7 membered saturated or partially unsaturated heterocyclic having 1-2 heteroatoms independently selected from nitrogen, oxygen, and

sulfur, and a 5-6 membered heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

each R^4 is independently hydrogen, a suitable prodrug, R^5 , halogen, -CN, -NO₂, -OR, -SR, -NR₂, -S(O)₂R, -S(O)₂NR₂, -S(O)R, -C(O)R, -C(O)OR, -C(O)NR₂, -C(O)N(R)OR, -OC(O)R, -OC(O)NR₂, -OP(O)R₂, -OP(O)(OR)₂, -OP(O)(OR)NR₂, -OP(O)(NR₂)₂-, -N(R)C(O)OR, -N(R)C(O)R, -N(R)C(O)NR₂, -N(R)S(O)₂R, -N(R)P(O)R₂, -N(R)P(O)(OR)₂, -N(R)P(O)(OR)NR₂, -N(R)P(O)(NR₂)₂-, -N(R)S(O)₂R, -Si(OR)₂R, -Si(OR)R₂, or -SiR₃;

each R^5 is independently an optionally substituted group selected from C₁₋₆ aliphatic, phenyl, a 4-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and a 5-6 membered heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

X^1 is O, S, or NR;

X^2 is -O-, -S-, -B(H)₂-, or a covalent bond;

X^3 is -O-, -S-, -Se-, or -N(R)-;

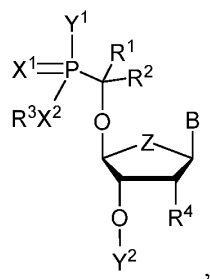
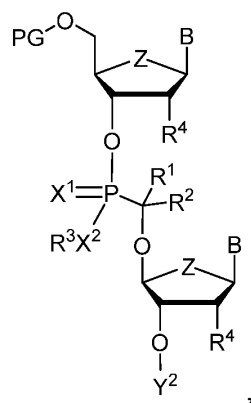
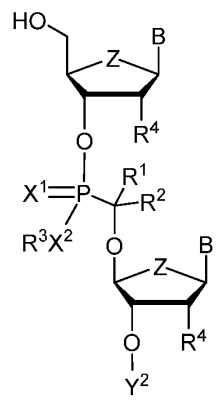
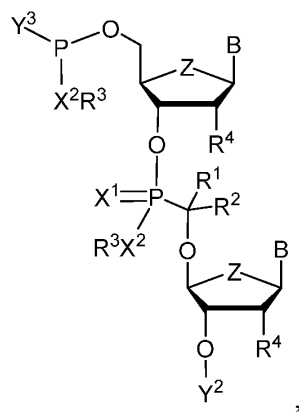
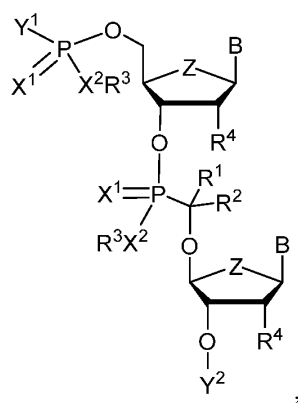
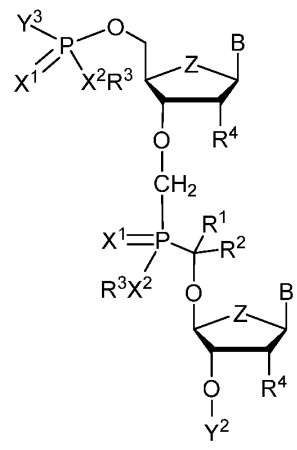
Y^1 is a linking group attaching to the 2'- or 3'-terminal of a nucleoside, a nucleotide, or an oligonucleotide;

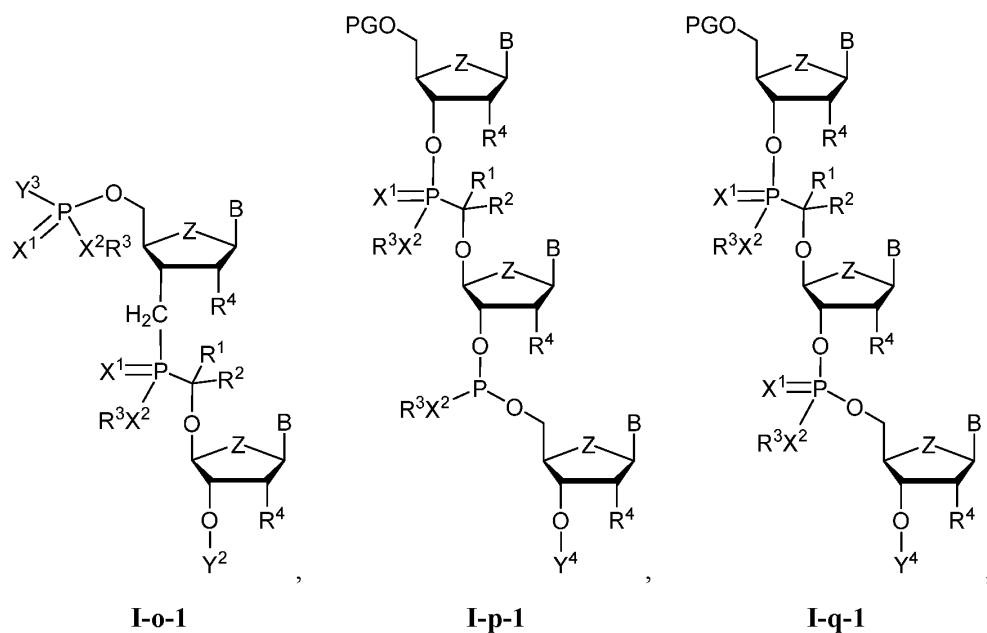
Y^2 is hydrogen, a protecting group, a phosphoramidite analogue, an internucleotide linking group attaching to the 4'- or 5'-terminal of a nucleoside, a nucleotide, or an oligonucleotide, or a linking group attaching to a solid support;

Z is -O-, -S-, -N(R)-, or -C(R)₂-; and

n is 0, 1, 2, 3, 4, or 5.

2. The nucleic acid or analogue thereof according to claim 1, wherein the 4'-O-methylene phosphonate internucleotide linkage is selected from any one of the representative formulae:

**I-a-1****I-b-1****I-g-1****I-h-1****I-i-1****I-n-1**

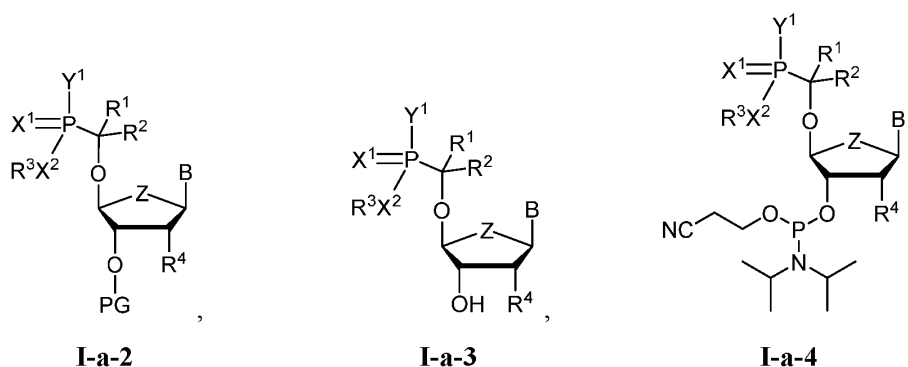


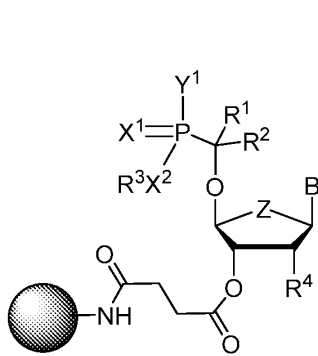
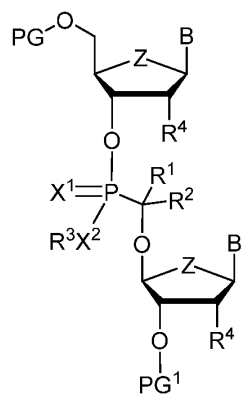
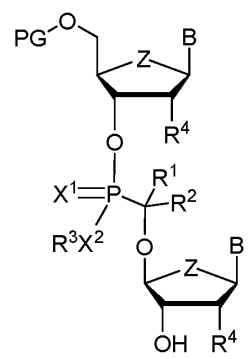
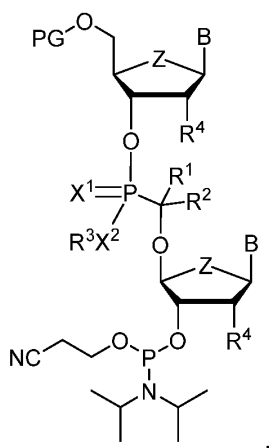
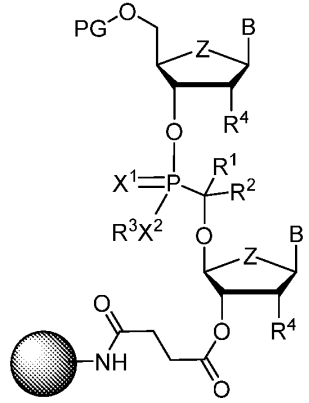
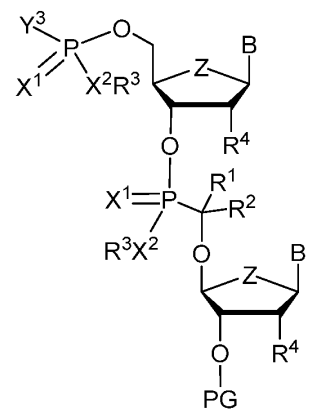
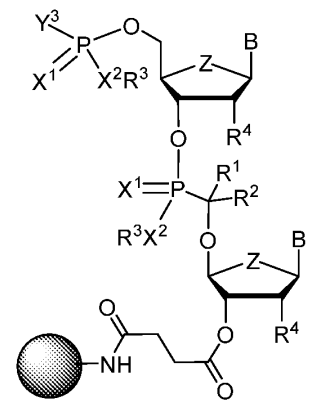
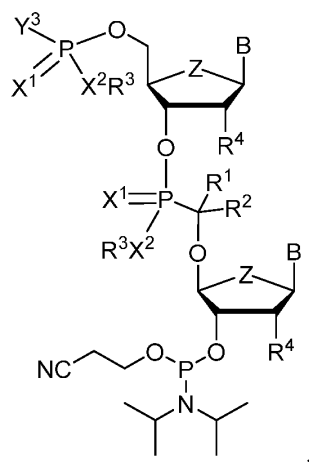
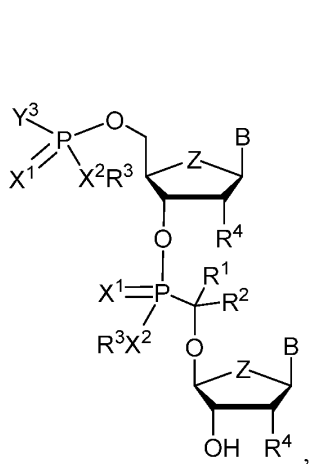
or a pharmaceutically acceptable salt thereof, wherein:

Y^3 is a linking group attaching to the 2'- or 3'-terminal of a nucleoside, a nucleotide, or an oligonucleotide; and

Y^4 is hydrogen, a protecting group, a phosphoramidite analogue, an internucleotide linking group attaching to the 4'- or 5'-terminal of a nucleoside, a nucleotide, or an oligonucleotide, or a linking group attaching to a solid support.

3. The nucleic acid or analogue thereof according to either claim 1 or claim 2, wherein the nucleic acid or analogue thereof is selected from any one of the following formulae:



**I-a-5****I-c-1****I-d-1****I-e-1****I-f-1****I-j-1**

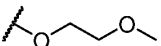
I-k-1

I-l-1

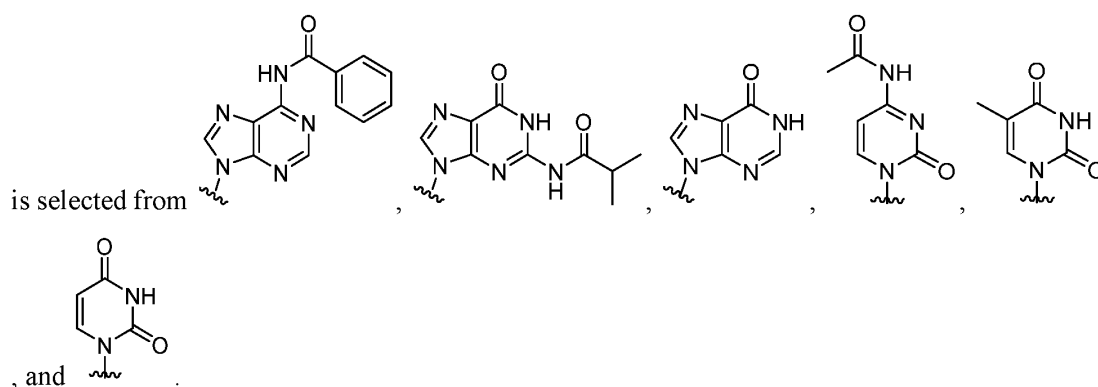
I-m-1

or a pharmaceutically acceptable salt thereof.

4. The nucleic acid or analogue thereof according to any one of claims 1-3, wherein R¹ is hydrogen and R² is hydrogen or methyl.

5. The nucleic acid or analogue thereof according to any one of claims 1-4, wherein each R⁴ is independently hydrogen, hydroxy, fluoro, methoxy, or .

6. The nucleic acid or analogue thereof according to any one of claims 1-5, wherein each B



7. The nucleic acid or analogue thereof according to any one of claims 1-6, wherein said nucleic acid or analogue thereof is selected from any one of those depicted in **Table 1**, or a pharmaceutically acceptable salt thereof.

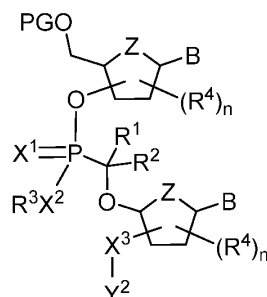
8. The nucleic acid or analogue thereof according to claim 1, wherein the nucleic acid or analogue thereof is a double-stranded RNAi inhibitor molecule comprising a first strand and a second strand, wherein the first strand is a sense strand and the second strand is an antisense strand.

9. The nucleic acid or analogue thereof according to claim 8, wherein the double stranded RNAi inhibitor molecule comprises a region of complementarity between the sense strand and the antisense strand of 15 to 45 nucleotides.

10. The nucleic acid or analogue thereof according to claim 9, wherein the region of complementarity between the sense strand and the antisense strand is 20 to 30 nucleotides.
11. The nucleic acid or analogue thereof according to claim 10, wherein the region of complementarity between the sense strand and the antisense strand is 21 to 26 nucleotides.
12. The nucleic acid or analogue thereof according to claim 9, wherein the region of complementarity between the sense strand and the antisense strand is 19 to 24 nucleotides.
13. The nucleic acid or analogue thereof according to claim 12, wherein the region of complementarity between the sense strand and the antisense strand is 19 to 21 nucleotides.
14. The nucleic acid or analogue thereof according to claim 8, wherein the double-stranded RNAi inhibitor molecule contains a tetraloop.
15. The nucleic acid or analogue thereof according to claim 1, wherein the nucleic acid or analogue thereof is a single stranded nucleic acid.
16. The nucleic acid or analogue thereof according to claim 15, wherein the single stranded nucleic acid is a single stranded RNAi inhibitor molecule.
17. The nucleic acid or analogue thereof according to claim 15, wherein the single-stranded nucleic acid is a conventional antisense nucleic acid, a ribozyme or an aptamer.
18. The nucleic acid or analogue thereof according to either claim 16 or claim 17, wherein the single stranded RNAi inhibitor molecule is 14-50 nucleotides in length.
19. The nucleic acid or analogue thereof according to claim 18, wherein the single stranded RNAi inhibitor molecule is about 16-30, 18-22, or 20-22 nucleotides in length.

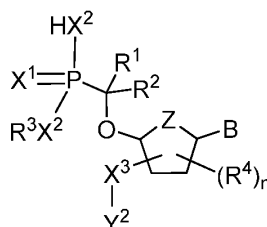
20. The nucleic acid or analogue thereof according to claim 1, wherein the nucleic acid or analogue thereof is a naked nucleic acid.
21. The nucleic acid or analogue thereof according to claim 1, further comprising at least one delivery agent, wherein the at least one delivery agent is conjugated to the nucleic acid or analogue thereof to facilitate transport of the nucleic acid or analogue thereof across an outer membrane of a cell.
22. The nucleic acid or analogue thereof according to claim 1, wherein the delivery agent is selected from the group consisting of carbohydrates, peptides, lipids, vitamins and antibodies.
23. The nucleic acid or analogue thereof according to claim 1, wherein the delivery agent is selected from N-Acetylgalactosamine (GalNAc), mannose-6-phosphate, galactose, oligosaccharide, polysaccharide, cholesterol, polyethylene glycol, folate, vitamin A, vitamin E, lithocholic acid and a cationic lipid.
24. A pharmaceutical composition comprising a nucleic acid or analogue thereof according to claim 1, and a pharmaceutically acceptable carrier, adjuvant, or vehicle.
25. A method for reducing expression of a target gene in a subject in need thereof, comprising administering the pharmaceutical composition of claim 24 to the subject in an amount sufficient to reduce expression of the target gene.
26. A method for treating cancer, a viral infection, or genetic disorder in a subject in need thereof, comprising administering the pharmaceutical composition of claim 24 to the subject in an amount sufficient to treat the cancer, viral infection, or genetic disorder.
27. The method according to either claim 24 or claim 25, wherein the administering comprises systemic administration.
28. A method for preparing a nucleic acid or analogue thereof comprising a 4'-O-methylene

phosphonate internucleotide linkage, wherein the 4'-O-methylene phosphonate internucleotide linkage is represented by formula **I-c**:

**I-c**

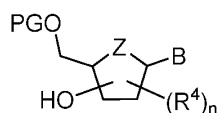
or a pharmaceutically acceptable salt thereof, comprising the steps:

- (a) providing a nucleic acid or analogue thereof of formula **A4**:

**A4**

or pharmaceutically acceptable salt thereof, and

- (b) condensing the nucleic acid or analogue thereof of formula **A4** with a nucleoside or analogue thereof of formula **A5**:

**A5**

to form the nucleic acid or analogue thereof comprising formula **I-b**, wherein:

each B is a nucleobase or hydrogen;

PG is a suitable hydroxyl protecting group;

R¹ and R² are independently hydrogen, halogen, R⁵, -CN, -S(O)R, -S(O)₂R, -Si(OR)₂R, -Si(OR)R₂, or -SiR₃, or:

R^1 and R^2 on the same carbon are taken together with their intervening atoms to form a 3-7 membered saturated or partially unsaturated ring having 0-3 heteroatoms, independently selected from nitrogen, oxygen, and sulfur;

each R is independently hydrogen, a suitable protecting group, or an optionally substituted group selected from C_{1-6} aliphatic, phenyl, a 4-7 membered saturated or partially unsaturated heterocyclic having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and a 5-6 membered heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or:

two R groups on the same atom are taken together with their intervening atoms to form a 4-7 membered saturated, partially unsaturated, or heteroaryl ring having 0-3 heteroatoms, independently selected from nitrogen, oxygen, silicon, and sulfur;

R^3 is hydrogen, a suitable protecting group, a suitable prodrug, or an optionally substituted group selected from C_{1-6} aliphatic, phenyl, a 4-7 membered saturated or partially unsaturated heterocyclic having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and a 5-6 membered heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

each R^4 is independently hydrogen, a suitable prodrug, R^5 , halogen, -CN, -NO₂, -OR, -SR, -NR₂, -S(O)₂R, -S(O)₂NR₂, -S(O)R, -C(O)R, -C(O)OR, -C(O)NR₂, -C(O)N(R)OR, -OC(O)R, -OC(O)NR₂, -OP(O)R₂, -OP(O)(OR)₂, -OP(O)(OR)NR₂, -OP(O)(NR₂)₂, -N(R)C(O)OR, -N(R)C(O)R, -N(R)C(O)NR₂, -N(R)S(O)₂R, -N(R)P(O)R₂, -N(R)P(O)(OR)₂, -N(R)P(O)(OR)NR₂, -N(R)P(O)(NR₂)₂, -N(R)S(O)₂R, -Si(OR)₂R, -Si(OR)R₂, or -SiR₃;

each R^5 is independently an optionally substituted group selected from C_{1-6} aliphatic, phenyl, a 4-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and a 5-6 membered heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

X^1 is O, S, or NR;

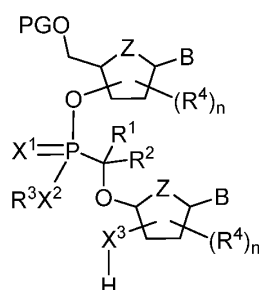
each X^2 is independently -O-, -S-, -B(H)₂-, or a covalent bond;

X^3 is -O-, -S-, -Se-, or -N(R)-;

Y^2 is hydrogen, a protecting group, a phosphoramidite analogue, an internucleotide linking group attaching to the 4'- or 5'-terminal of a nucleoside, a nucleotide, or an oligonucleotide, or a linking group attaching to a solid support;
 each Z is independently -O-, -S-, -N(R)-, or -C(R)₂-; and
 each n is independently 0, 1, 2, 3, 4, or 5.

29. The method of claim 27, wherein Y^2 is a protecting group.

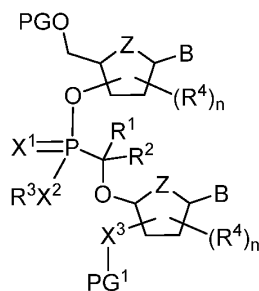
30. The method of claim 29, further comprising the steps of preparing a nucleic acid of formula **I-d**:



I-d

or a pharmaceutically acceptable salt thereof, comprising the steps:

(a) providing a nucleic acid or analogue thereof comprising of formula **I-c**:



I-c

or pharmaceutically acceptable salt thereof, and

(b) deprotecting the nucleic acid or analogue thereof comprising formula **I-c** to form the nucleic acid or analogue thereof comprising formula **I-d**, wherein:

each B is a nucleobase or hydrogen;

PG is a suitable hydroxyl protecting group;

PG¹ is a protecting group;

R¹ and R² are independently hydrogen, halogen, R⁵, -CN, -S(O)R, -S(O)₂R, -Si(OR)₂R, -Si(OR)R₂, or -SiR₃, or:

R¹ and R² on the same carbon are taken together with their intervening atoms to form a 3-7 membered saturated or partially unsaturated ring having 0-3 heteroatoms, independently selected from nitrogen, oxygen, and sulfur;

each R is independently hydrogen, a suitable protecting group, or an optionally substituted group selected from C₁₋₆ aliphatic, phenyl, a 4-7 membered saturated or partially unsaturated heterocyclic having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and a 5-6 membered heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or:

two R groups on the same atom are taken together with their intervening atoms to form a 4-7 membered saturated, partially unsaturated, or heteroaryl ring having 0-3 heteroatoms, independently selected from nitrogen, oxygen, silicon, and sulfur;

R³ is hydrogen, a suitable protecting group, a suitable prodrug, or an optionally substituted group selected from C₁₋₆ aliphatic, phenyl, a 4-7 membered saturated or partially unsaturated heterocyclic having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and a 5-6 membered heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

each R⁴ is independently hydrogen, a suitable prodrug, R⁵, halogen, -CN, -NO₂, -OR, -SR, -NR₂, -S(O)₂R, -S(O)₂NR₂, -S(O)R, -C(O)R, -C(O)OR, -C(O)NR₂, -C(O)N(R)OR, -OC(O)R, -OC(O)NR₂, -OP(O)R₂, -OP(O)(OR)₂, -OP(O)(OR)NR₂, -OP(O)(NR₂)₂, -N(R)C(O)OR, -N(R)C(O)R, -N(R)C(O)NR₂, -N(R)S(O)₂R, -N(R)P(O)R₂, -N(R)P(O)(OR)₂, -N(R)P(O)(OR)NR₂, -N(R)P(O)(NR₂)₂, -N(R)S(O)₂R, -Si(OR)₂R, -Si(OR)R₂, or -SiR₃;

each R⁵ is independently an optionally substituted group selected from C₁₋₆ aliphatic, phenyl, a 4-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and a 5-6 membered heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

X¹ is O, S, or NR;

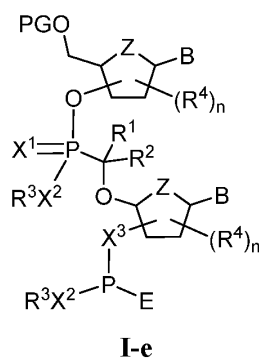
each X^2 is independently -O-, -S-, -B(H)₂-, or a covalent bond;

X^3 is -O-, -S-, -Se-, or -N(R)-;

each Z is independently -O-, -S-, -N(R)-, or -C(R)₂-; and

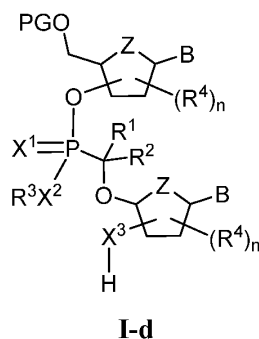
each n is independently 0, 1, 2, 3, 4, or 5.

31. The method of claim 30, further comprising the steps of preparing a nucleic acid or analogue thereof of formula **I-e**:



or a pharmaceutical acceptable salt thereof, comprising the steps:

(a) providing a nucleic acid or analogue thereof of formula **I-d**:



(b) reacting the nucleic acid or analogue thereof of formula **I-d** with a P(III) forming reagent to form the nucleic acid or analogue thereof of formula **I-e**, wherein:

each B is a nucleobase or hydrogen;

PG is a suitable hydroxyl protecting group;

R^1 and R^2 are independently hydrogen, halogen, R^5 , -CN, -S(O)R, -S(O)₂R, -Si(OR)₂R, -Si(OR)R₂, or -SiR₃, or:

R^1 and R^2 on the same carbon are taken together with their intervening atoms to form a 3-7 membered saturated or partially unsaturated ring having 0-3 heteroatoms, independently selected from nitrogen, oxygen, and sulfur;

each R is independently hydrogen, a suitable protecting group, or an optionally substituted group selected from C_{1-6} aliphatic, phenyl, a 4-7 membered saturated or partially unsaturated heterocyclic having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and a 5-6 membered heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or:

two R groups on the same atom are taken together with their intervening atoms to form a 4-7 membered saturated, partially unsaturated, or heteroaryl ring having 0-3 heteroatoms, independently selected from nitrogen, oxygen, silicon, and sulfur;

R^3 is hydrogen, a suitable protecting group, a suitable prodrug, or an optionally substituted group selected from C_{1-6} aliphatic, phenyl, a 4-7 membered saturated or partially unsaturated heterocyclic having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and a 5-6 membered heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

each R^4 is independently hydrogen, a suitable prodrug, R^5 , halogen, -CN, -NO₂, -OR, -SR, -NR₂, -S(O)₂R, -S(O)₂NR₂, -S(O)R, -C(O)R, -C(O)OR, -C(O)NR₂, -C(O)N(R)OR, -OC(O)R, -OC(O)NR₂, -OP(O)R₂, -OP(O)(OR)₂, -OP(O)(OR)NR₂, -OP(O)(NR₂)₂, -N(R)C(O)OR, -N(R)C(O)R, -N(R)C(O)NR₂, -N(R)S(O)₂R, -N(R)P(O)R₂, -N(R)P(O)(OR)₂, -N(R)P(O)(OR)NR₂, -N(R)P(O)(NR₂)₂, -N(R)S(O)₂R, -Si(OR)₂R, -Si(OR)R₂, or -SiR₃;

each R^5 is independently an optionally substituted group selected from C_{1-6} aliphatic, phenyl, a 4-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and a 5-6 membered heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

E is a halogen or -NR₂;

X^1 is O, S, or NR;

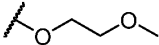
each X^2 is independently -O-, -S-, -B(H)₂-, or a covalent bond;

X^3 is -O-, -S-, -Se-, or -N(R)-;

each Z is independently -O-, -S-, -N(R)-, or -C(R)₂-; and

each n is independently 0, 1, 2, 3, 4, or 5.

32. The method according to any one of claims 28-31, wherein each R¹ is hydrogen and R² is hydrogen or methyl.

33. The method according to any one of claims 28-32, wherein each R⁴ is independently hydrogen, hydroxy, fluoro, methoxy, or .

34. The method according to any one of claims 28-33, wherein each B is selected from

