LIGAND-2'-MODIFIED NUCLEIC ACIDS, SYNTHESIS THEREOF AND INTERMEDIATE COMPOUNDS THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application no. 62/894,071, filed August 30, 2019, the content of which is incorporated by reference herein in its entirety.

TECHNICAL FIELD OF THE INVENTION

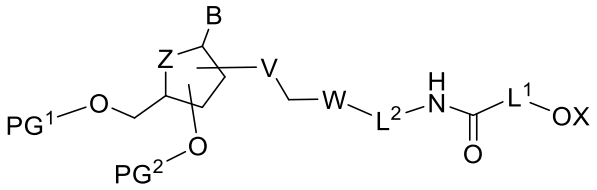
[0002] The present invention relates to method for synthesizing compounds useful as potent and stable RNA interference agents, derivatives thereof, and intermediates thereto.

BACKGROUND OF THE INVENTION

[0003] Double-stranded RNA (dsRNA) agents possessing strand lengths of 25 to 35 nucleotides have been described as effective inhibitors of target gene expression in mammalian cells (Rossi et al., U.S. Patent Publication Nos. 2005/0244858 and 2005/0277610). dsRNA agents of such length are believed to be processed by the Dicer enzyme of the RNA interference (RNAi) pathway, leading such agents to be termed "Dicer substrate siRNA" ("DsiRNA") agents. Certain modified structures of DsiRNA agents were previously described (Rossi et al., U.S. Patent Publication No. 2007/0265220).

SUMMARY

[0003a] In an aspect of the present disclosure, there is provided a method for preparing a compound of formula **D-a**:



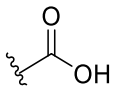
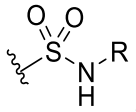
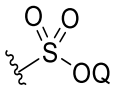
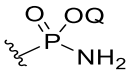
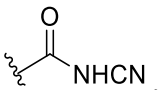
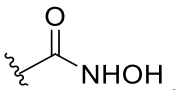
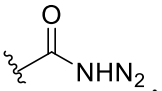
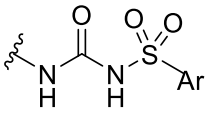
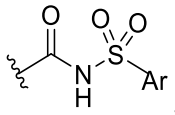
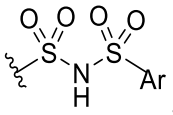
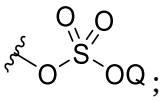
D-a

or a salt thereof, wherein:

PG^1 and PG^2 are independently hydrogen or a suitable hydroxyl protecting group;

B is a nucleobase or hydrogen;

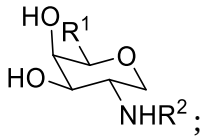
each L^1 and L^2 are independently a bivalent moiety selected from alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, or substituted alkynyl, wherein one or more methylenes can be interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OY, S, S(OY), $SO_2(Y)$, (C=O)OY, NY_2, NH, and NH—(C=OY);

Y is independently selected from H, C_1-C_6 alkanyl, C_1-C_6 alkenyl or aryl, including , , , , , , , , , , and ;

each R is independently selected from hydrogen, alkyl, alkenyl, aromatic, heterocycle, substituted alkyl, and substituted alkenyl;

Q is H or a pharmaceutically acceptable salt, C_1-C_6 alkanyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, aryl, heteroaryl, $(CH_2)_m$-aryl or $(CH_2)_m$-heteroaryl where m is 1-10 and any of the aryl or heteroaryl rings may be substituted with one to three independently selected Cl, F, CF_3, C_1-C_8 alkoxy, NO_2, C_1-C_6 alkanyl, C_1-C_6 alkenyl, aryl or OY, C(O)OY, NY_2 or C(O)NHY;

V and W are independently -O-, -S-, or -NR-;

X is a ligand selected from GalNAc, D-mannose, L-galactose, D-arabinose, L-fucose, polyols, and ;

R^1 is selected from CF_3, alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, and substituted alkynyl;

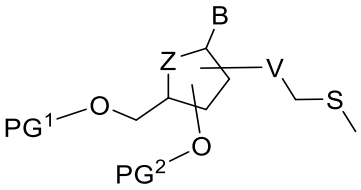
R^2 is selected from one or more methylenes interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OR^3, S, $S(OR^3)$, $SO_2(R^3)$, $(C=O)OR^3$, NY_2, NH, and $NH(C=OR^3)$;

R^3 is H, C_1-C_6 alkanyl, C_1-C_6 alkenyl, or aryl; and

Z is $-CH_2-$, -O-, -S-, or -NR-,

comprising the steps of:

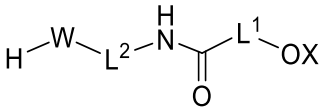
(a) providing a compound of formula **F-1-a**:



F-1-a

or a salt thereof, and

(b) reacting said fragment compound of formula **F-1-a** with a fragment compound of formula **F-6**:

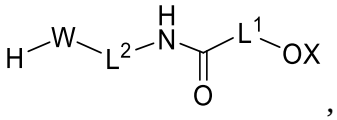


F-6

or a salt thereof,

to provide the compound of formula **D-a**.

[0003b] In another aspect of the present disclosure, there is provided a compound, wherein the compound is of formula **F-6:**

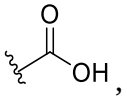


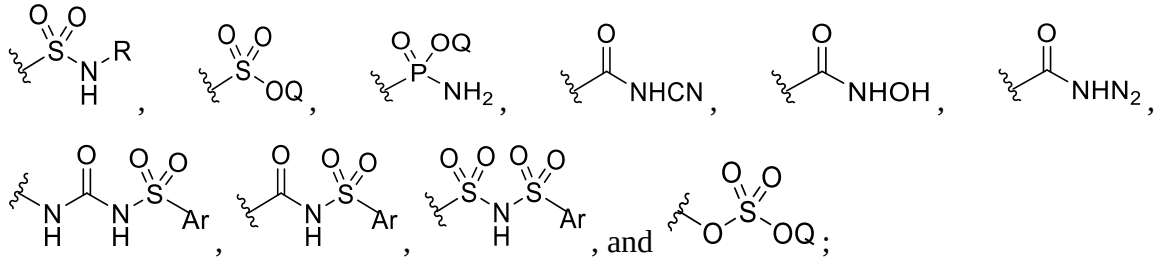
,

F-6

or a pharmaceutically acceptable salt thereof, wherein:

each L^1 and L^2 are independently a bivalent moiety selected from alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, or substituted alkynyl, wherein one or more methylenes can be interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OY, S, S(OY), $SO_2(Y)$, (C=O)OY, NY_2, NH, and NH—(C=OY);

Y is independently selected from H, C_1-C_6 alkanyl, C_1-C_6 alkenyl or aryl, including ,

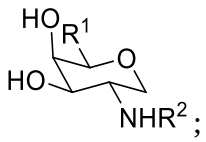


each R is independently selected from hydrogen, alkyl, alkenyl, aromatic, heterocycle, substituted alkyl, and substituted alkenyl;

Q is H or a pharmaceutically acceptable salt, C_1-C_6 alkanyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, aryl, heteroaryl, $(CH_2)_m$-aryl or $(CH_2)_m$-heteroaryl where m is 1-10 and any of the aryl or heteroaryl rings may be substituted with one to three independently selected Cl, F, CF_3, C_1-C_8 alkoxy, NO_2, C_1-C_6 alkanyl, C_1-C_6 alkenyl, aryl or OY, C(O)OY, NY_2 or C(O)NHY;

W is -O-, -S-, or -NR-;

X is a ligand selected from GalNAc, D-mannose, L-galactose, D-arabinose, L-fucose, polyols, and

;

R^1 is selected from CF_3, alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, and substituted alkynyl;

R^2 is selected from one or more methylenes interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OR^3, S, $S(OR^3)$, $SO_2(R^3)$, $(C=O)OR^3$, NY_2, NH, and $NH(C=OR^3)$; and

R^3 is H, C_1-C_6 alkanyl, C_1-C_6 alkenyl, or aryl.

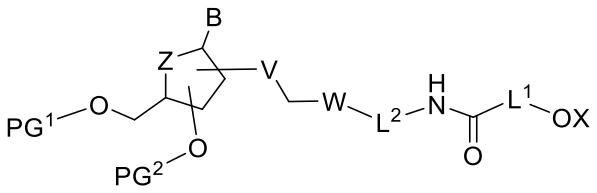
DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0004] The methods and intermediates of the present disclosure are useful for preparing various analogues of compounds as described in, e.g. Brown et al., U.S. Patent Publication No. 2017/0305956, the entirety of which is herein incorporated by reference. The compounds provided herein are useful as pharmaceutical agents for the treatment of disease. In certain embodiments, a compound of formula **A** is generally prepared by the assembly of three fragments **F-1**, **F-2**, and **F-3** as shown by **Scheme 1** set forth below:

CLAIMS

We claim:

1. A method for preparing a compound of formula **D-a**:



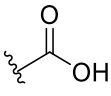
D-a

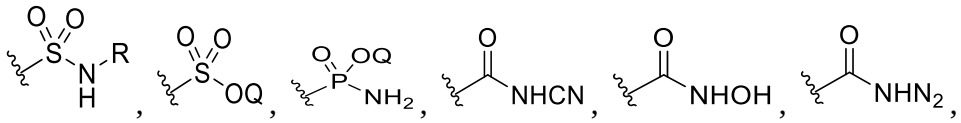
or a salt thereof, wherein:

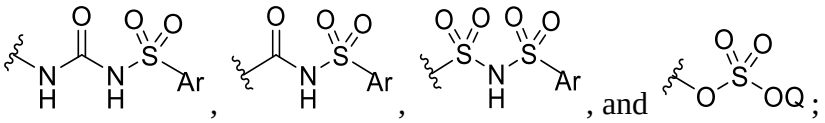
PG^1 and PG^2 are independently hydrogen or a suitable hydroxyl protecting group;

B is a nucleobase or hydrogen;

each L^1 and L^2 are independently a bivalent moiety selected from alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, or substituted alkynyl, wherein one or more methylenes can be interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OY, S, S(OY), $SO_2(Y)$, (C=O)OY, NY_2, NH, and NH—(C=OY);

Y is independently selected from H, C_1-C_6 alkanyl, C_1-C_6 alkenyl or aryl, including ,





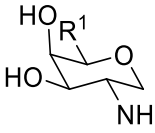
, and ;

each R is independently selected from hydrogen, alkyl, alkenyl, aromatic, heterocycle, substituted alkyl, and substituted alkenyl;

Q is H or a pharmaceutically acceptable salt, C_1-C_6 alkanyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, aryl, heteroaryl, $(CH_2)_m$-aryl or $(CH_2)_m$-heteroaryl where m is 1-10 and any of the aryl or heteroaryl rings may be substituted with one to three independently selected Cl, F, CF_3, C_1-C_8 alkoxy, NO_2, C_1-C_6 alkanyl, C_1-C_6 alkenyl, aryl or OY, C(O)OY, NY_2 or C(O)NHY;

V and W are independently -O-, -S-, or -NR-;

X is a ligand selected from GalNAc, D-mannose, L-galactose, D-arabinose, L-fucose, polyols,



and ;

R^1 is selected from CF_3, alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, and substituted alkynyl;

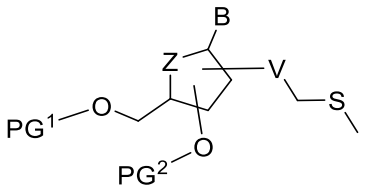
R^2 is selected from one or more methylenes interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OR^3, S, $S(OR^3)$, $SO_2(R^3)$, $(C=O)OR^3$, NY_2, NH, and $NH(C=OR^3)$;

R^3 is H, C_1-C_6 alkanyl, C_1-C_6 alkenyl, or aryl; and

Z is $-CH_2-$, -O-, -S-, or -NR-,

comprising the steps of:

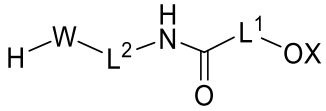
(a) providing a compound of formula **F-1-a**:



F-1-a

or a salt thereof, and

(b) reacting said fragment compound of formula **F-1-a** with a fragment compound of formula **F-6**:

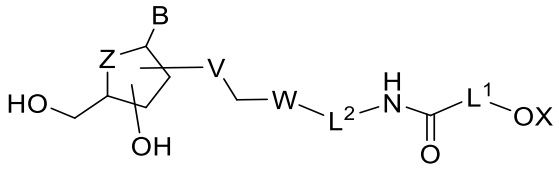


F-6

or a salt thereof,

to provide the compound of formula **D-a**.

2. The method of claim 1, further comprising the step of preparing a compound of formula **C-a**:

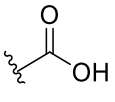


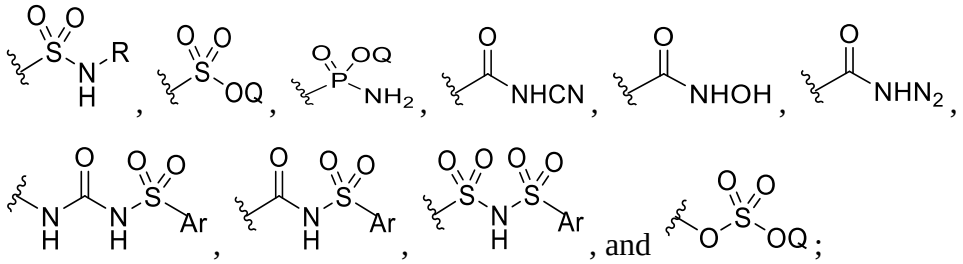
C-a

or a pharmaceutically acceptable salt thereof, wherein:

B is a nucleobase or hydrogen;

each L^1 and L^2 are independently a bivalent moiety selected from alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, or substituted alkynyl, wherein one or more methylenes can be interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OY, S, S(OY), $SO_2(Y)$, (C=O)OY, NY_2, NH, and NH—(C=OY);

Y is independently selected from H, C_1-C_6 alkanyl, C_1-C_6 alkenyl or aryl, including ,

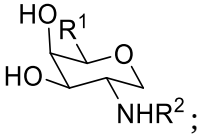


each R is independently selected from hydrogen, alkyl, alkenyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl;

Q is H or a pharmaceutically acceptable salt, C_1-C_6 alkanyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, aryl, heteroaryl, $(CH_2)_m$-aryl or $(CH_2)_m$-heteroaryl where m is 1-10 and any of the aryl or heteroaryl rings may be substituted with one to three independently selected Cl, F, CF_3, C_1-C_8 alkoxy, NO_2, C_1-C_6 alkanyl, C_1-C_6 alkenyl, aryl or OY, C(O)OY, NY_2 or C(O)NHY;

V and W are independently -O-, -S-, or -NR-;

X is a ligand selected from GalNAc, D-mannose, L-galactose, D-arabinose, L-fucose, polyols,

and ;

R^1 is selected from CF_3, alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, and substituted alkynyl;

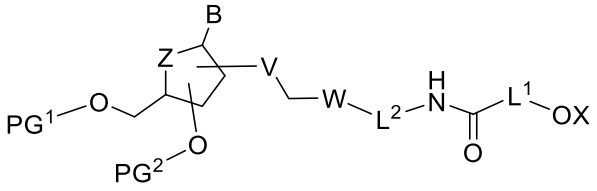
R^2 is selected from one or more methylenes interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OR^3, S, $S(OR^3)$, $SO_2(R^3)$, $(C=O)OR^3$, NY_2, NH, and $NH(C=OR^3)$;

R^3 is H, C_1-C_6 alkanyl, C_1-C_6 alkenyl, or aryl; and

Z is $-CH_2-$, -O-, -S-, or -NR-,

comprising the steps of:

(a) providing a compound of formula **D-a**:

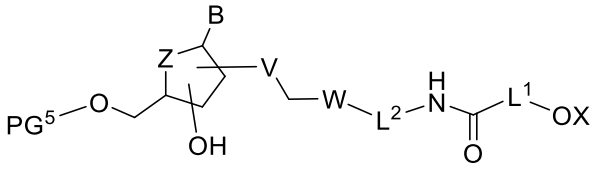


D-a

or a pharmaceutically acceptable salt thereof, and

(b) deprotecting said compound of formula **D-a** to form a compound of formula **C-a**.

3. The method of claim 2, further comprising the step of preparing a compound of formula **B-a**:



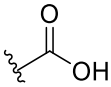
B-a

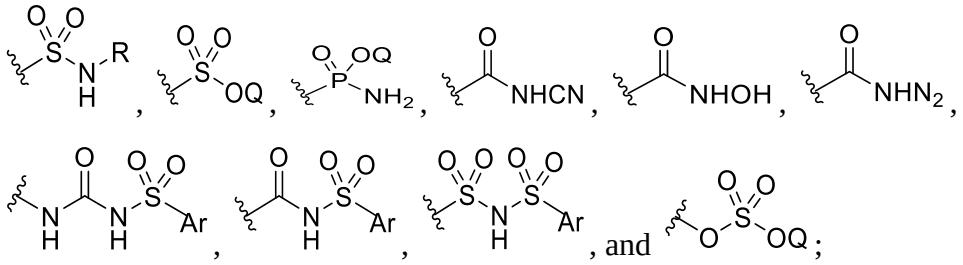
or a pharmaceutically acceptable salt thereof, wherein:

PG^5 is hydrogen or a suitable hydroxyl protecting group;

B is a nucleobase or hydrogen;

each L^1 and L^2 are independently a bivalent moiety selected from alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, or substituted alkynyl, wherein one or more methylenes can be interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OY, S, S(OY), $SO_2(Y)$, (C=O)OY, NY_2, NH, and NH—(C=OY);

Y is independently selected from H, C_1-C_6 alkanyl, C_1-C_6 alkenyl or aryl, including ,

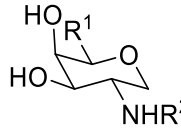


each R is independently selected from hydrogen, alkyl, alkenyl, aromatic, heterocycle, substituted alkyl, and substituted alkenyl;

Q is H or a pharmaceutically acceptable salt, C_1-C_6 alkanyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, aryl, heteroaryl, $(CH_2)_m$-aryl or $(CH_2)_m$-heteroaryl where m is 1-10 and any of the aryl or heteroaryl rings may be substituted with one to three independently selected Cl, F, CF_3, C_1-C_8 alkoxy, NO_2, C_1-C_6 alkanyl, C_1-C_6 alkenyl, aryl or OY, C(O)OY, NY_2 or C(O)NHY;

V and W are independently -O-, -S-, or -NR-;

X is a ligand selected from GalNAc, D-mannose, L-galactose, D-arabinose, L-fucose, polyols,

and ;

R^1 is selected from CF_3, alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, and substituted alkynyl;

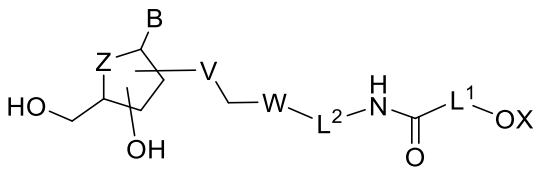
R^2 is selected from one or more methylenes interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OR^3, S, $S(OR^3)$, $SO_2(R^3)$, $(C=O)OR^3$, NY_2, NH, and $NH(C=OR^3)$;

R^3 is H, C_1-C_6 alkanyl, C_1-C_6 alkenyl, or aryl; and

Z is $-CH_2-$, -O-, -S-, or -NR-,

comprising the steps of:

(a) providing a compound of formula **C-a**:

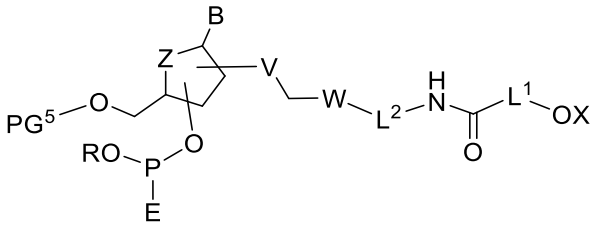


C-a

or a pharmaceutically acceptable salt thereof, and

(b) protecting said compound of formula **C-a** with a suitable protecting group to form a compound of formula **B-a**.

4. The method of claim 3, further comprising the steps of preparing a compound of formula **A-a**:



A-a

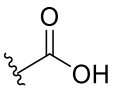
or a pharmaceutically acceptable salt thereof, wherein:

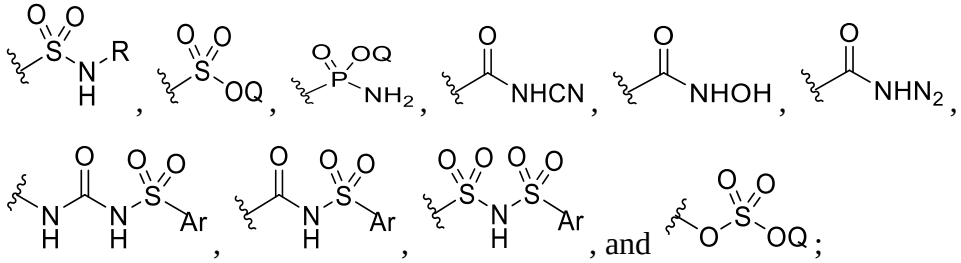
PG^5 is hydrogen or a suitable hydroxyl protecting group;

B is a nucleobase or hydrogen;

E is a halogen or NR_2;

each L^1 and L^2 are independently a bivalent moiety selected from alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, or substituted alkynyl, wherein one or more methylenes can be interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OY, S, S(OY), $SO_2(Y)$, (C=O)OY, NY_2, NH, and NH—(C=OY);

Y is independently selected from H, C_1-C_6 alkanyl, C_1-C_6 alkenyl or aryl, including ,



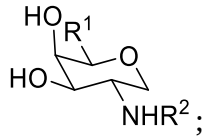
each R is independently selected from hydrogen, alkyl, alkenyl, aromatic, heterocycle, substituted alkyl, and substituted alkenyl, or:

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

Q is H or a pharmaceutically acceptable salt, C_1-C_6 alkanyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, aryl, heteroaryl, $(CH_2)_m$-aryl or $(CH_2)_m$-heteroaryl where m is 1-10 and any of the aryl or heteroaryl rings may be substituted with one to three independently selected Cl, F, CF_3, C_1-C_8 alkoxy, NO_2, C_1-C_6 alkanyl, C_1-C_6 alkenyl, aryl or OY, C(O)OY, NY_2 or C(O)NHY;

V and W are independently -O-, -S-, or -NR-;

X is a ligand selected from GalNAc, D-mannose, L-galactose, D-arabinose, L-fucose, polyols,

and 

;

R^1 is selected from CF_3, alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, and substituted alkynyl;

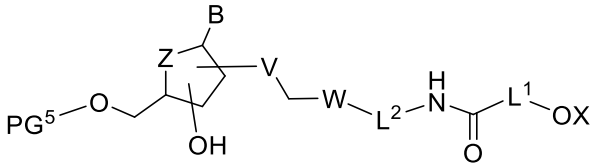
R^2 is selected from one or more methylenes interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OR^3, S, $S(OR^3)$, $SO_2(R^3)$, $(C=O)OR^3$, NY_2, NH, and $NH(C=OR^3)$;

R^3 is H, C_1-C_6 alkanyl, C_1-C_6 alkenyl, or aryl; and

Z is $-CH_2-$, -O-, -S-, or -NR-,

comprising the steps of:

(a) providing a compound of formula **B-a**:

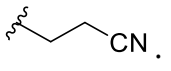


B-a

or a pharmaceutically acceptable salt thereof, and

(b) reacting said compound of formula **B-a** with a P(III) forming reagent to form a compound of formula **A-a**.

5. The method of claim 4, wherein E is NR_2.

6. The method of claim 5, wherein R is selected from isopropyl and .

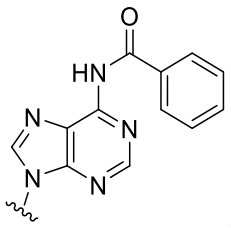
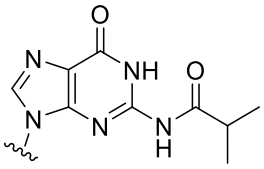
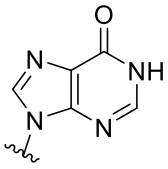
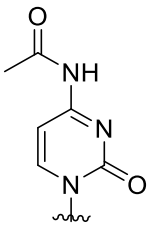
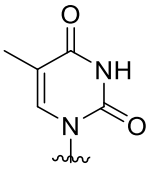
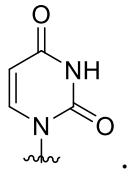
7. The method of claim 1, wherein PG^1 and PG^2 are taken together with their intervening atoms to form a cyclic diol protecting group.

8. The method of claim 7, wherein the cyclic diol protecting group comprises 1,1,3,3-tetraisopropylidisiloxanylidene.

9. The method of any one of claims 3-4, wherein PG^5 is 4,4'-dimethyoxytrityl.

10. The method of any one of claims 1-9, wherein B is a purine or pyrimidine base.

11. The method of claim 10, wherein the purine or pyrimidine base is G, A, or C comprising a protecting group.

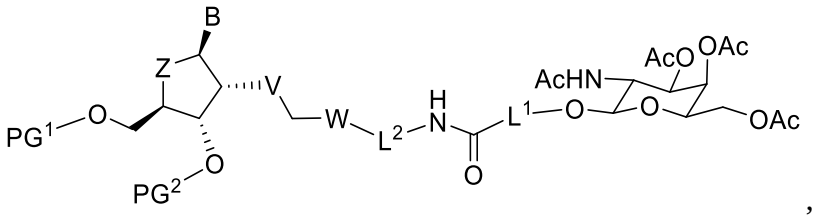
12. The method of claim 10, wherein purine or pyrimidine base is selected from , , , , , and .

13. The method of any one of claims 1-12, wherein V is –O–.

14. The method of any one of claims 1-13, wherein W is –O–.

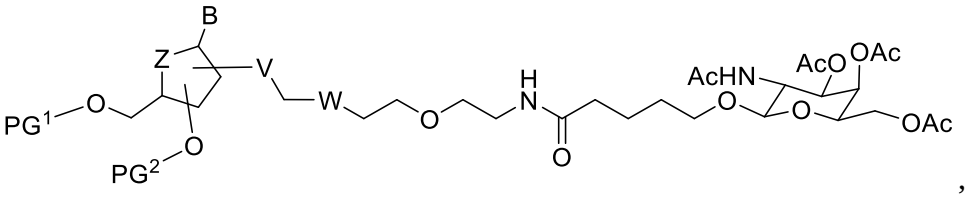
15. The method of any one of claims 1-14, wherein Z is –O–.

16. The method of claim 1, wherein the compound of formula **D-a** is a compound of any one of the following formulae:



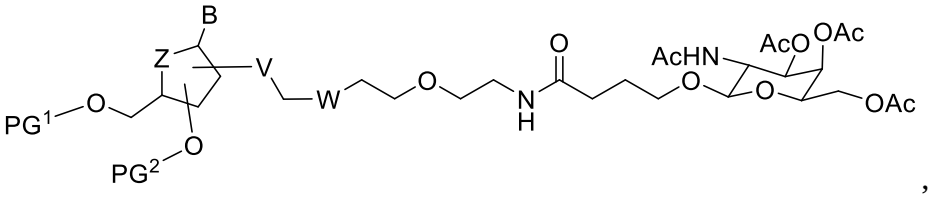
,

D-b



,

D-c

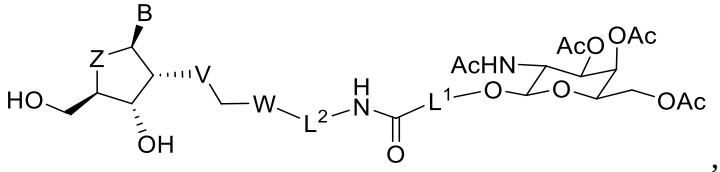


,

D-d

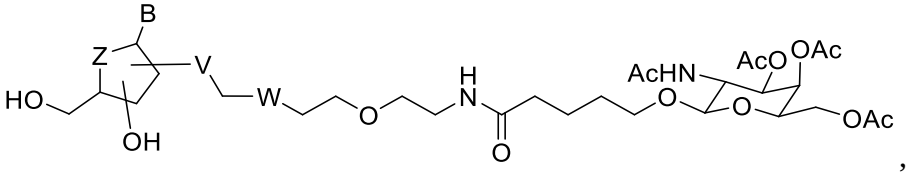
or a pharmaceutically acceptable salt thereof.

17. The method of claim 2, wherein the compound of formula **C-a** is a compound of any one of the following formulae:



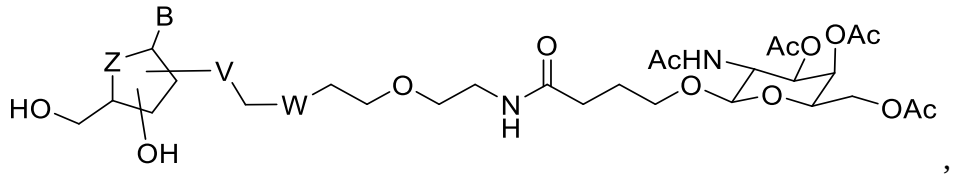
,

C-b



,

C-c

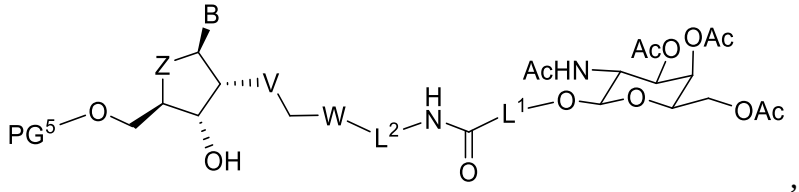


,

C-d

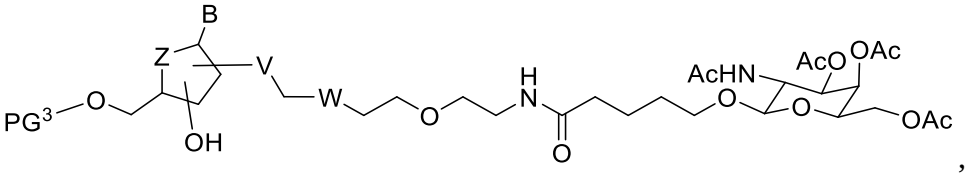
or a pharmaceutically acceptable salt thereof.

18. The method of claim 3, wherein the compound of formula **B-a** is a compound of any one of the following formulae:



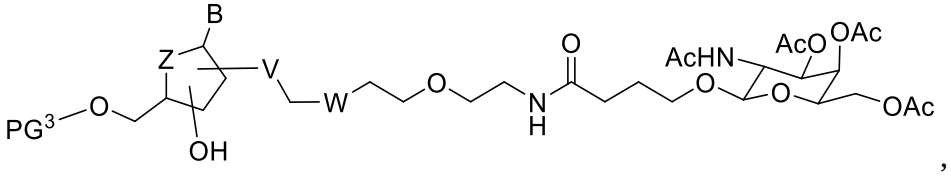
,

B-b



,

B-c

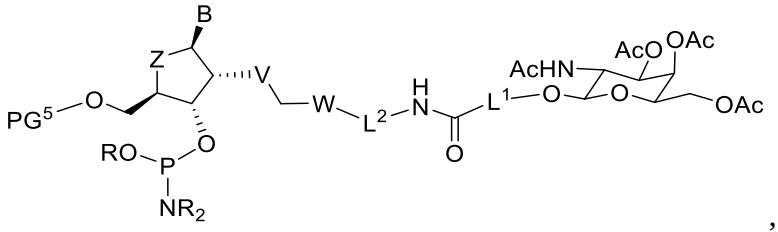


,

B-d

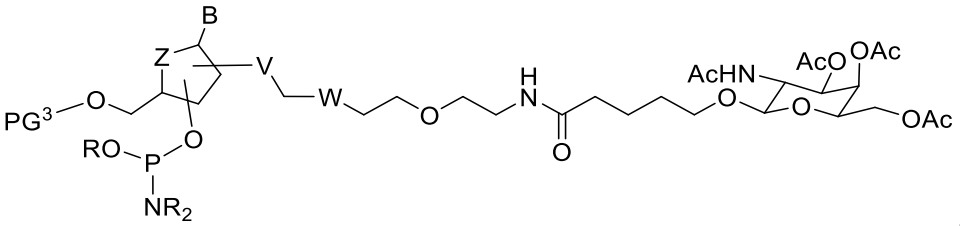
or a pharmaceutically acceptable salt thereof.

19. The method of claim 4, wherein the compound of formula **A-a** is a compound of any one of the following formulae:



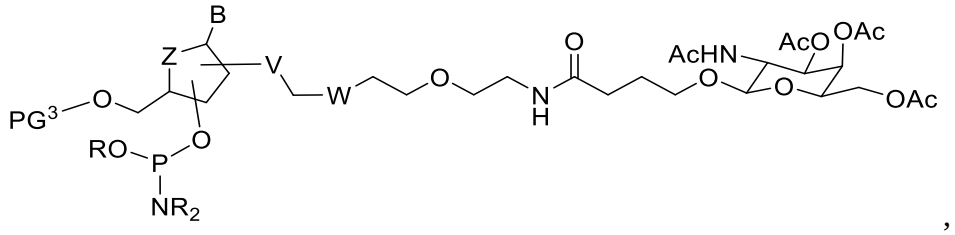
,

A-b



,

A-c

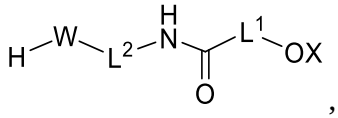


,

A-d

or a pharmaceutically acceptable salt thereof.

20. A compound, wherein the compound is of formula **F-6:**

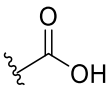


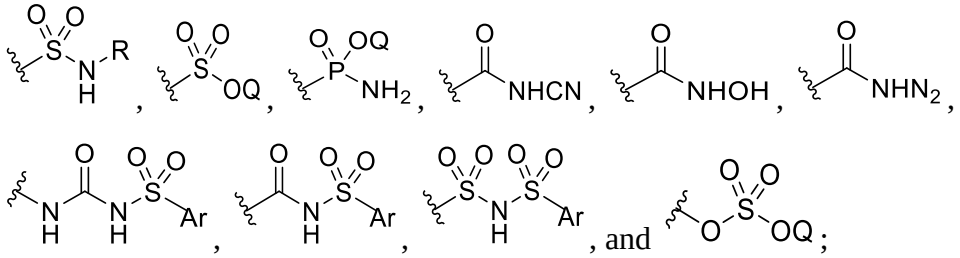
,

F-6

or a pharmaceutically acceptable salt thereof, wherein:

each L^1 and L^2 are independently a bivalent moiety selected from alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, or substituted alkynyl, wherein one or more methylenes can be interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OY, S, S(OY), $SO_2(Y)$, (C=O)OY, NY_2, NH, and NH—(C=OY);

Y is independently selected from H, C_1-C_6 alkanyl, C_1-C_6 alkenyl or aryl, including ,



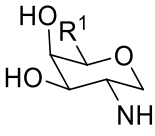
;

each R is independently selected from hydrogen, alkyl, alkenyl, aromatic, heterocycle, substituted alkyl, and substituted alkenyl;

Q is H or a pharmaceutically acceptable salt, C_1-C_6 alkanyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, aryl, heteroaryl, $(CH_2)_m$-aryl or $(CH_2)_m$-heteroaryl where m is 1-10 and any of the aryl or heteroaryl rings may be substituted with one to three independently selected Cl, F, CF_3, C_1-C_8 alkoxy, NO_2, C_1-C_6 alkanyl, C_1-C_6 alkenyl, aryl or OY, C(O)OY, NY_2 or C(O)NHY;

W is -O-, -S-, or -NR-;

X is a ligand selected from GalNAc, D-mannose, L-galactose, D-arabinose, L-fucose, polyols,



and ;

R^1 is selected from CF_3, alkyl, alkenyl, alkynyl, aromatic, heterocycle, substituted alkyl, substituted alkenyl, and substituted alkynyl;

R^2 is selected from one or more methylenes interrupted or terminated by one or more of P(O)H, $P(O_2)$, $P(O_4)$, polyethylenegylcol (PEG), OR^3, S, $S(OR^3)$, $SO_2(R^3)$, $(C=O)OR^3$, NY_2, NH, and $NH(C=OR^3)$; and

R^3 is H, C_1-C_6 alkanyl, C_1-C_6 alkenyl, or aryl.