

CLAIMS

1. A double stranded RNAi oligonucleotide for reducing ketohexokinase (*KHK*) expression, the oligonucleotide comprising an antisense strand and a sense strand, wherein the antisense strand and the sense strand form a duplex region, wherein the antisense strand comprises a region of complementarity to a *KHK* mRNA target sequence of any one of SEQ ID NOs: 4-387 and wherein the region of complementarity is at least 15 contiguous nucleotides in length, or a pharmaceutically acceptable salt thereof, wherein preferably the sense strand comprises a sequence set forth in any one of SEQ ID NOs: 4-387 and/or the antisense strand comprises a sequence set forth in any one of SEQ ID NOs: 388-771.
2. A double stranded RNAi oligonucleotide for inhibiting expression of *KHK*, wherein said double stranded RNAi oligonucleotide comprises a sense strand and an antisense strand forming a duplex region, wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:4-387 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO: 388-771, or a pharmaceutically acceptable salt thereof, wherein preferably the sense strand is 18 to 36 nucleotides in length and/or the antisense strand is 15-30 nucleotides in length.
3. A double stranded RNAi (dsRNAi) oligonucleotide for reducing or inhibiting ketohexokinase (*KHK*) expression, the oligonucleotide comprising:
- (i) an antisense strand of 19-30 nucleotides in length, wherein the antisense strand comprises a nucleotide sequence comprising a region of complementarity to a *KHK* mRNA target sequence, wherein the region of complementarity is selected from SEQ ID NOs: 948-953; and
- (ii) a sense strand of 19-50 nucleotides in length comprising a region of complementarity to the antisense strand, wherein the antisense and sense strands are separate strands which form an asymmetric duplex region having an overhang of 1-4 nucleotides at the 3' terminus of the antisense strand.
4. The RNAi oligonucleotide of any one of claims 1 or 3, wherein the region of complementarity comprised by the antisense strand is at least 19 contiguous nucleotides in length, preferably 19 nucleotides.
5. The RNAi oligonucleotide of any one of claims 1-4, wherein the duplex region is at least 20 nucleotides in length, preferably 20 nucleotides.
6. The RNAi oligonucleotide of any one of claims 1-5, wherein the sense strand comprises at its 3' end a stem-loop set forth as: S1-L-S2, wherein S1 is complementary to S2, and

wherein L forms a loop between S1 and S2 of 3 to 5 nucleotides in length,
preferably the stem-loop comprises the sequence 5'-GCAGCCGAAAGGCUGC-3' (SEQ ID NO: 871).

7. The RNAi oligonucleotide of any one of claims 1-6,
5 wherein at least one nucleotide of the oligonucleotide is conjugated to one or more targeting ligands,
wherein preferably each targeting ligand comprises an N-acetylgalactosamine (GalNAc) moiety,
wherein more preferably the one or more targeting ligands is conjugated to one or more nucleotides of the loop
according to claim 6.
- 10 8. The RNAi oligonucleotide of any one of claims 1-7,
wherein the overhang is 2 nucleotides in length,
preferably selected from AA, GG, AG, and GA,
more preferably the overhang is GG.
- 15 9. The RNAi oligonucleotide of any one of claims 1-8,
wherein all the nucleotides of the oligonucleotide are modified,
preferably 10-15%, 10%, 11%, 12%, 13%, 14% or 15% of the nucleotides of the sense strand comprise a 2'-fluoro
modification
and/or about 25-35%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34% or 35% of the nucleotides of the
20 antisense strand comprise a 2'-fluoro modification.
10. The RNAi oligonucleotide of any one of claims 1-9,
wherein the oligonucleotide comprises at least one phosphorothioate linkage,
preferably between positions 1 and 2, between positions 2 and 3, and between positions 3 and 4,
25 wherein positions are numbered 1-4 from 5' to 3'.
11. The RNAi oligonucleotide of any one of claims 1-10,
wherein the 4'-carbon of the sugar of the 5'-terminal nucleotide of the antisense strand comprises a phosphate
analog,
30 preferably a 4'-phosphate analog comprising 5'-methoxyphosphonate-4'-oxy.
12. The RNAi oligonucleotide of any one of claims 1-11,
wherein the antisense strand is 22 nucleotides in length and/or
wherein the sense strand is 36 nucleotides in length.
- 35 13. The RNAi oligonucleotide of any one of claims 1-12,
wherein the sense and antisense strands comprise the nucleotide sequences set forth in
(a) SEQ ID NOs: 887 and 913, respectively, or

- (b) SEQ ID NOs: 891 and 917, respectively, or
- (c) SEQ ID NOs: 892 and 918, respectively, or
- (d) SEQ ID NOs: 894 and 920, respectively, or
- (e) SEQ ID NOs: 897 and 923, respectively, or
- 5 (f) SEQ ID NOs: 909 and 936, respectively.

14. A double stranded RNAi oligonucleotide (dsRNAi) for inhibiting expression of KHK, wherein said dsRNAi comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a KHK RNA transcript, e.g. KHK mRNA, wherein

10

- (a) the sense strand comprises the sequence and all of the modifications of 5'-mG-S-mA-mA-mG-mA-mG-mA-fA-fG-fC-fA-mG-mA-mU-mC-mC-mU-mG-mU-mA-mG-mC-mA-mG-mC-mC-mG-[adema-GalNAc]-[adema-GalNAc]-[adema-GalNAc]-mG-mG-mC-mU-mG-mC-3' (SEQ ID NO: 775), and the antisense strand comprises the sequence and all of the modifications of 5'-[MePhosphonate-4O-mU]-S-fA-S-fC-fA-fG-mG-fA-mU-mC-fU-mG-mC-mU-fU-mC-mU-mC-mU-mU-mC-S-mG-S-mG-3' (SEQ ID NO: 820); or
- 15

- (b) the sense strand comprises the sequence and all of the modifications of 5'-mC-S-mA-mG-mA-mU-mG-mU-fG-fU-fC-fU-mG-mC-mU-mA-mC-mA-mG-mA-mA-mG-mC-mA-mG-mC-mC-mG-[adema-GalNAc]-[adema-GalNAc]-[adema-GalNAc]-mG-mG-mC-mU-mG-mC-3' (SEQ ID NO: 779), and
- 20 the antisense strand comprises the sequence and all of the modifications of 5'-[MePhosphonate-4O-mU]-S-fU-S-fC-S-fU-fG-mU-fA-mG-mC-fA-mG-mA-mC-fA-mC-mA-mU-mC-mU-mG-S-mG-S-mG-3' (SEQ ID NO: 824); or

- (c) the sense strand comprises the sequence and all of the modifications of 5'-mG-S-mA-mC-mU-mU-mU-mG-fA-fG-fA-fA-mG-mG-mU-mU-mG-mA-mU-mC-mA-mG-mC-mA-mG-mC-mC-mG-[adema-GalNAc]-[adema-GalNAc]-[adema-GalNAc]-mG-mG-mC-mU-mG-mC-3' (SEQ ID NO: 780), and
- 25 the antisense strand comprises the sequence and all of the modifications of 5'-[MePhosphonate-4O-mU]-S-fG-S-fA-S-fU-fC-mA-fA-mC-mC-fU-mU-mC-mU-fC-mA-mA-mA-mG-mU-mC-S-mG-S-mG-3' (SEQ ID NO: 825); or

- (d) the sense strand comprises the sequence and all of the modifications of 5'-mU-S-mU-mU-mG-mA-mG-mA-fA-fG-fG-fU-mU-mG-mA-mU-mC-mU-mG-mA-mA-mG-mC-mA-mG-mC-mC-mG-[adema-GalNAc]-[adema-GalNAc]-[adema-GalNAc]-mG-mG-mC-mU-mG-mC-3' (SEQ ID NO: 782), and
- 30 the antisense strand comprises the sequence and all of the modifications of 5'-[MePhosphonate-4O-mU]-S-fU-S-fC-S-fA-fG-mA-fU-mC-mA-fA-mC-mC-mU-fU-mC-mU-mC-mA-mA-mA-S-mG-S-mG-3' (SEQ ID NO: 827); or

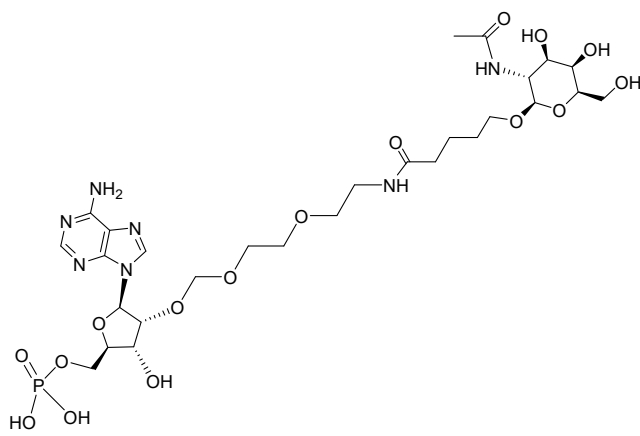
- (e) the sense strand comprises the sequence and all of the modifications of 5'-mU-S-mG-mU-mU-mU-mG-mU-fC-fA-fG-fC-mA-mA-mA-mG-mA-mU-mG-mU-mA-mG-mC-mA-mG-mC-mC-mG-[adema-GalNAc]-[adema-GalNAc]-[adema-GalNAc]-mG-mG-mC-mU-mG-mC-3' (SEQ ID NO: 785), and
- 35

the antisense strand comprises the sequence and all of the modifications of 5'-[MePhosphonate-4O-mU]-S-fA-S-fC-fA-fU-mC-fU-mU-mU-fG-mC-mU-mG-fA-mC-mA-mA-mA-mC-mA-S-mG-S-mG-3' (SEQ ID NO: 830); or

- (f) the sense strand comprises the sequence and all of the modifications of 5'-mG-S-mC-mA-mG-mG-mA-mA-fG-fC-fA-fC-mU-mG-mA-mG-mA-mU-mU-mC-mA-mG-mC-mA-mG-mC-mC-mG-[ademA-GalNAc]-[ademA-GalNAc]-[ademA-GalNAc]-mG-mG-mC-mU-mG-mC-3' (SEQ ID NO: 804), and

the antisense strand comprises the sequence and all of the modifications of 5'-[MePhosphonate-4O-mU]-S-fG-S-fA-S-fA-fU-mC-fU-mC-mA-fG-mU-mG-mC-fU-mU-mC-mC-mU-mG-mC-S-mG-S-mG-3' (SEQ ID NO: 849);

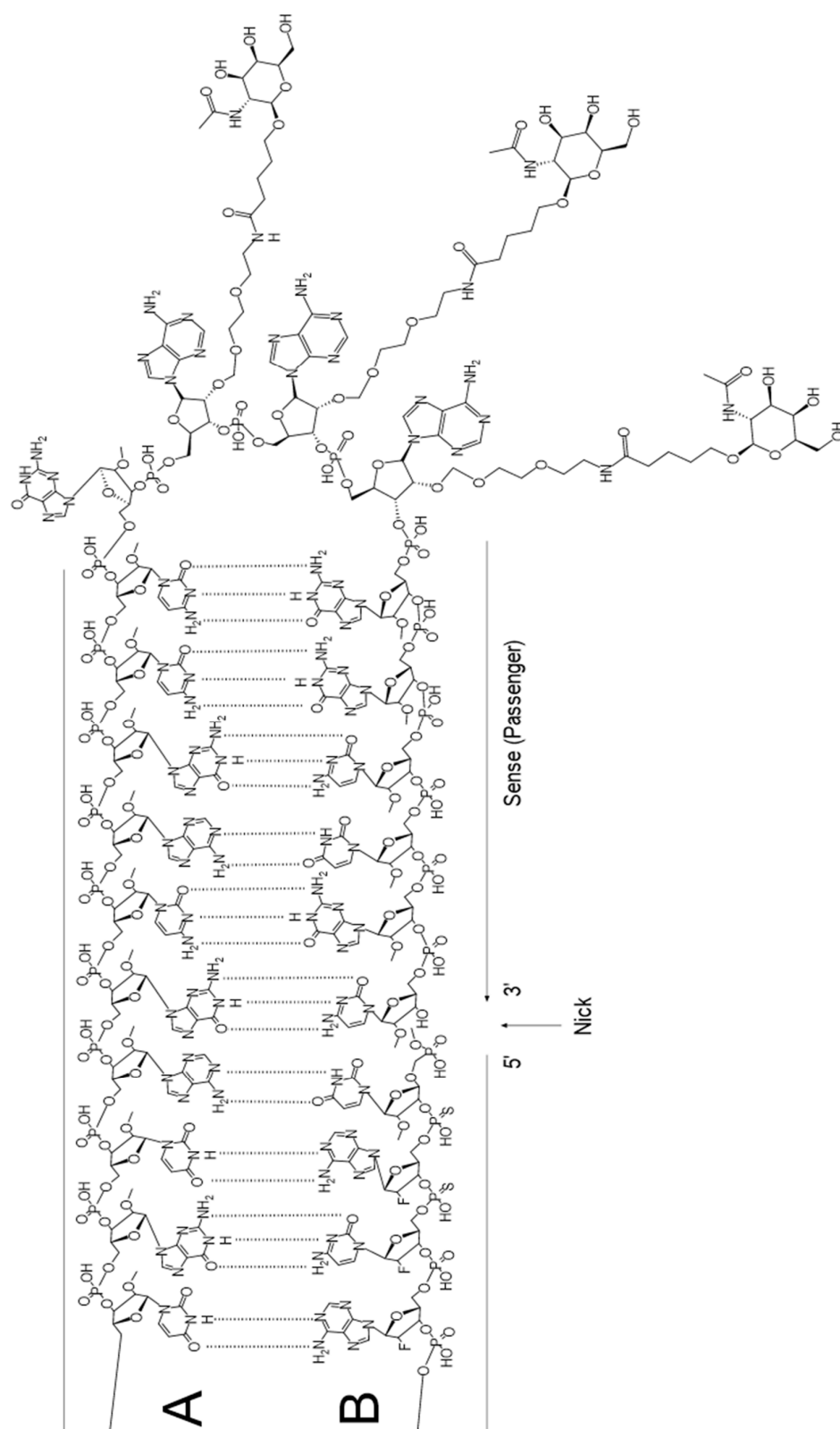
- wherein mC, mA, mG, mU=2'-OMe ribonucleosides; fA, fC, fG, fU=2'F ribonucleosides; "-"=phosphodiester linkage, "-S-"=phosphorothioate linkage, and wherein ademA-GalNAc =

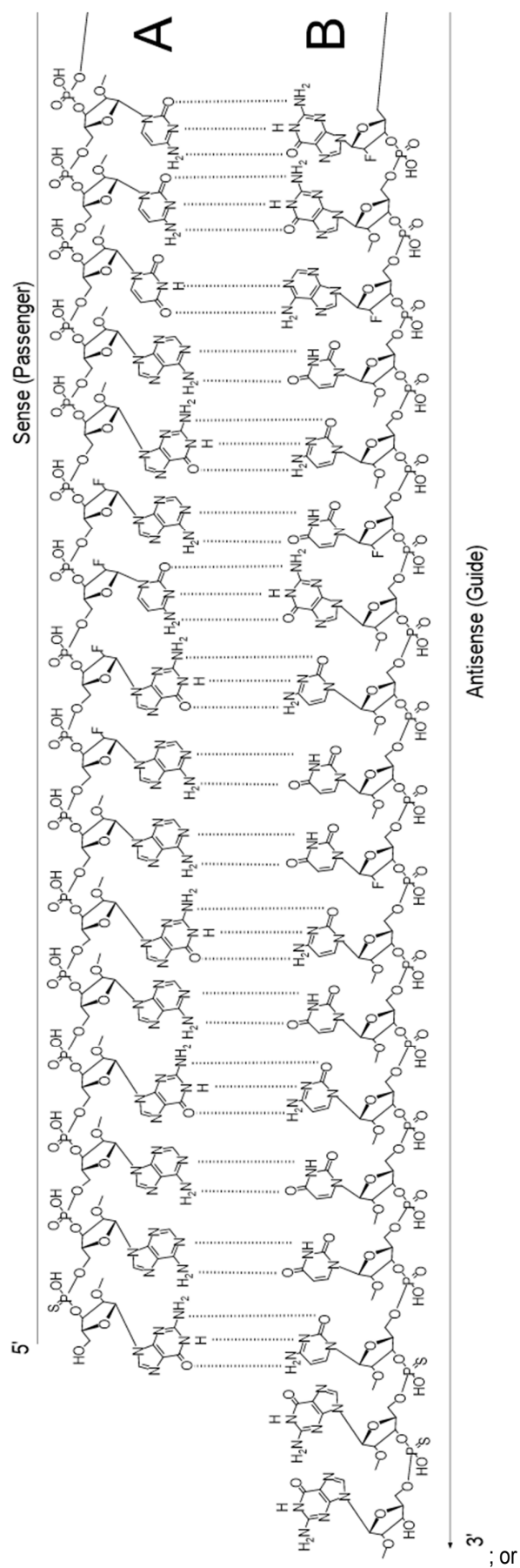


or a pharmaceutically acceptable salt thereof.

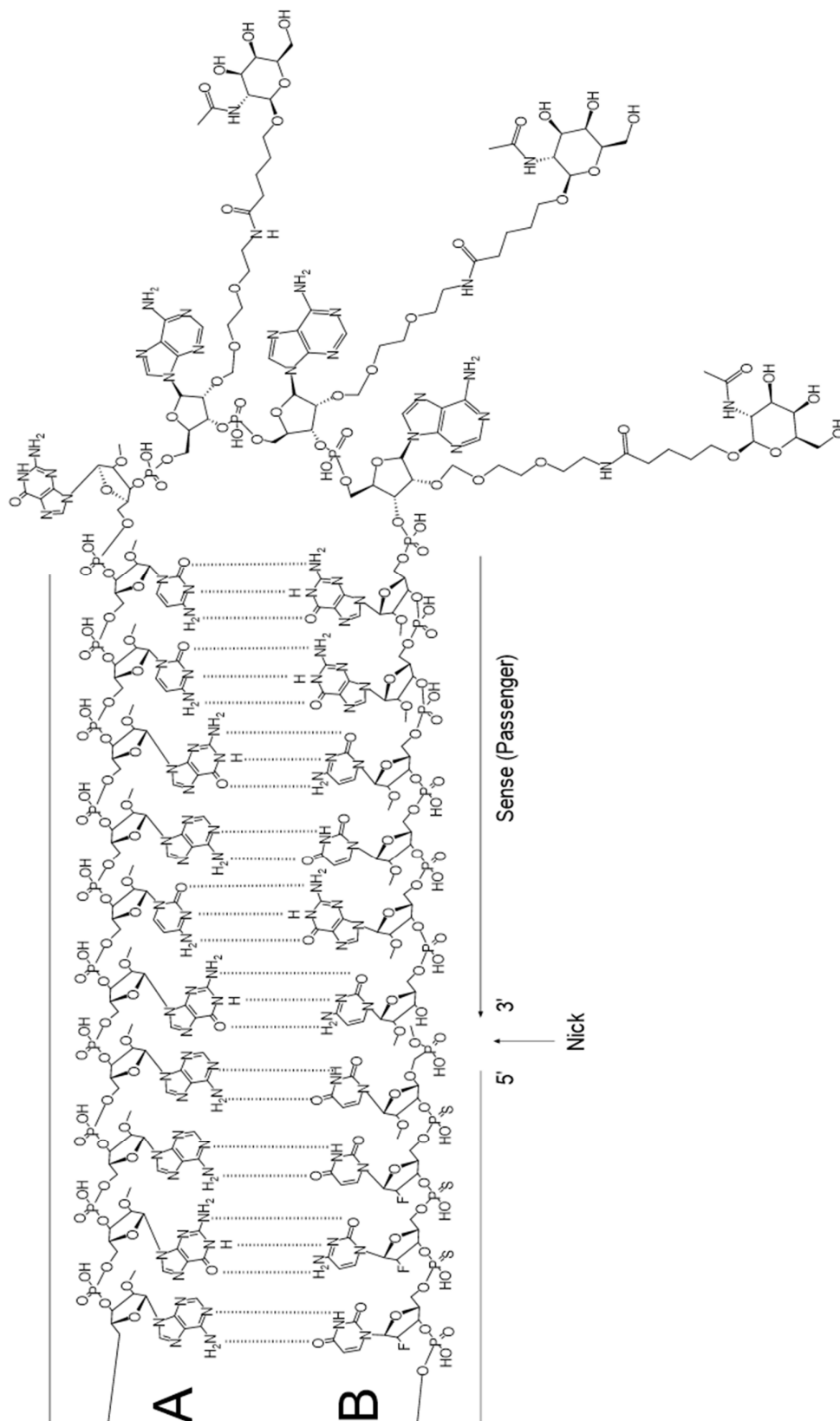
15

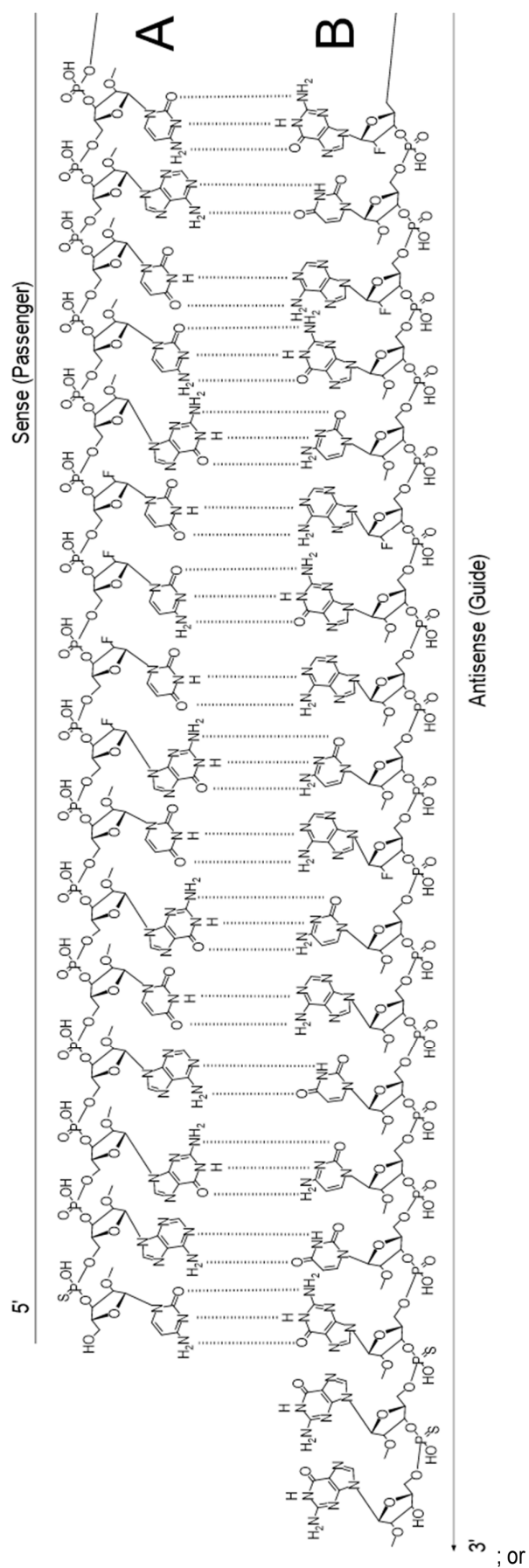
15. A double stranded RNAi oligonucleotide (dsRNAi) for inhibiting expression of KHK, wherein said dsRNAi comprises (a) a sense strand comprising SEQ ID NO: 775 and an antisense strand comprising SEQ ID NO: 820, the antisense strand comprising a region of complementarity to a KHK RNA transcript, e.g. KHK mRNA, wherein said dsRNAi is in the form of a conjugate having the structure of:



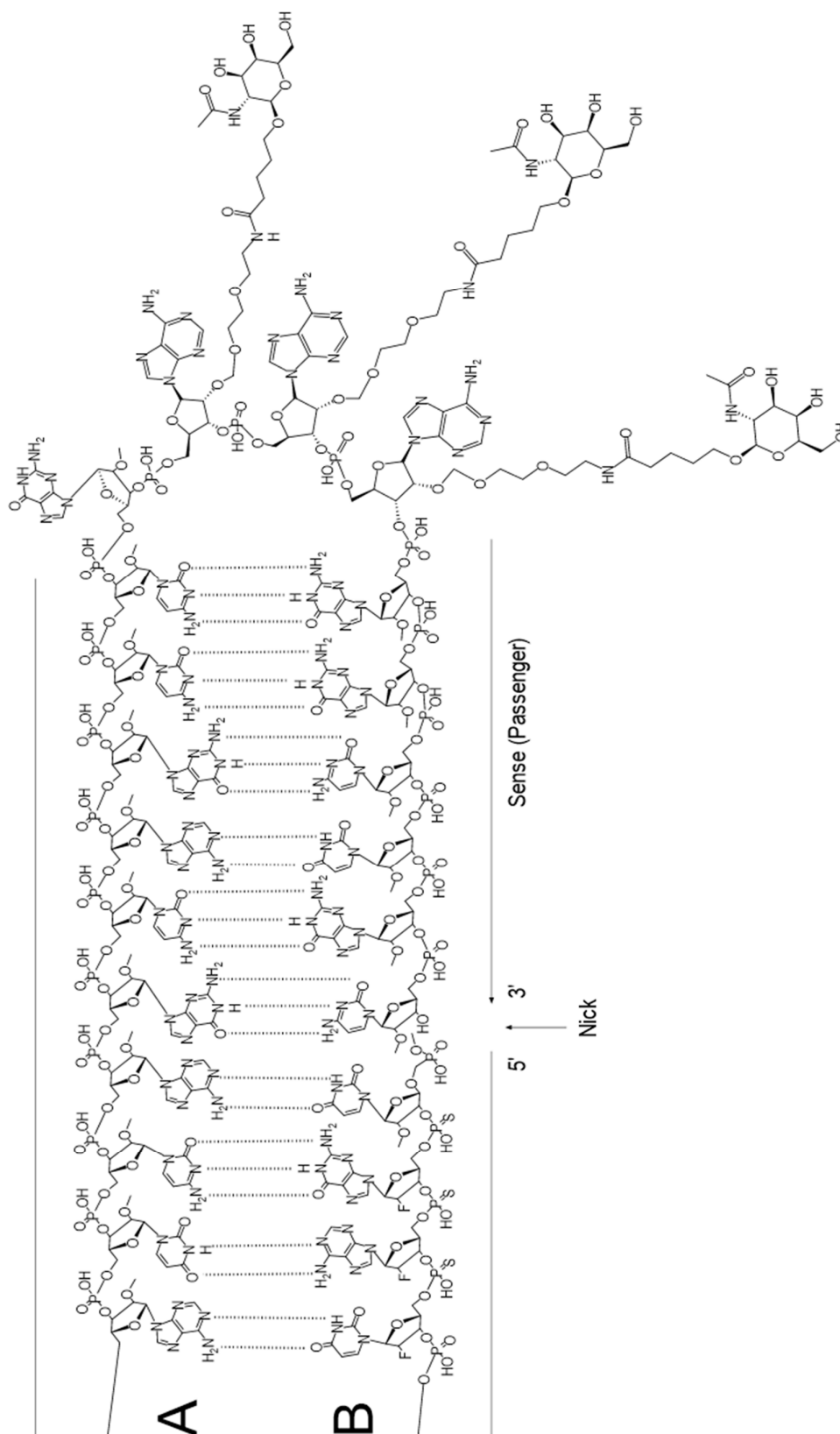


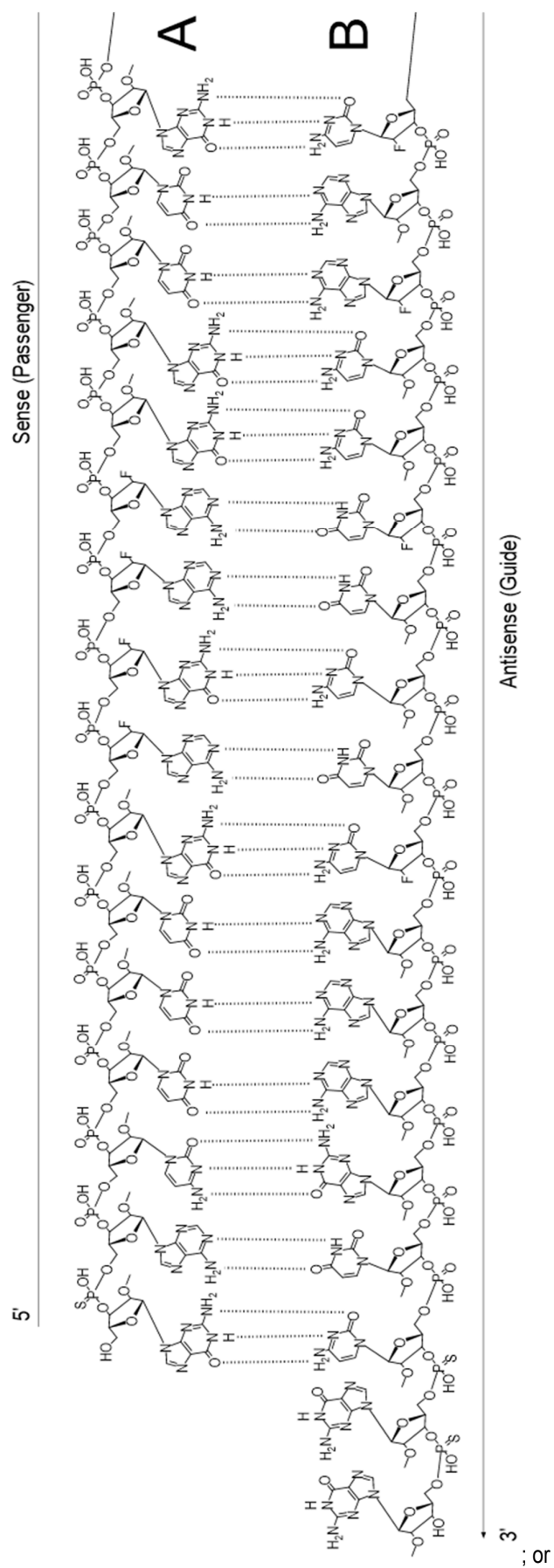
(b) a sense strand comprising SEQ ID NO: 779 and an antisense strand comprising SEQ ID NO: 824, the antisense strand comprising a region of complementarity to a KHK RNA transcript, e.g. KHK mRNA, wherein said dsRNAi is in the form of a conjugate having the structure of:



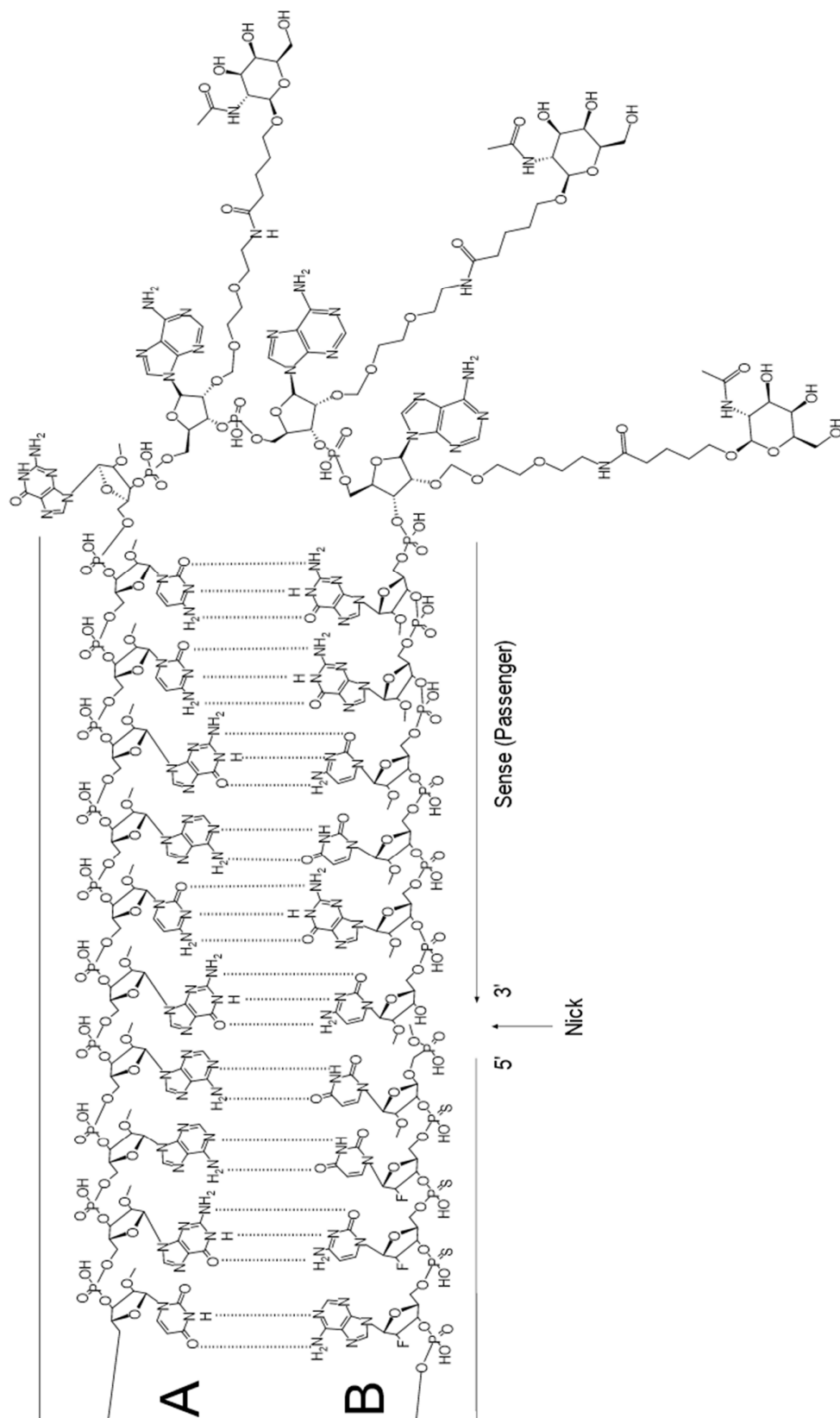


(c) a sense strand comprising SEQ ID NO: 780 and an antisense strand comprising SEQ ID NO: 825, the antisense strand comprising a region of complementarity to a KHK RNA transcript, e.g. KHK mRNA, wherein said dsRNAi is in the form of a conjugate having the structure of:



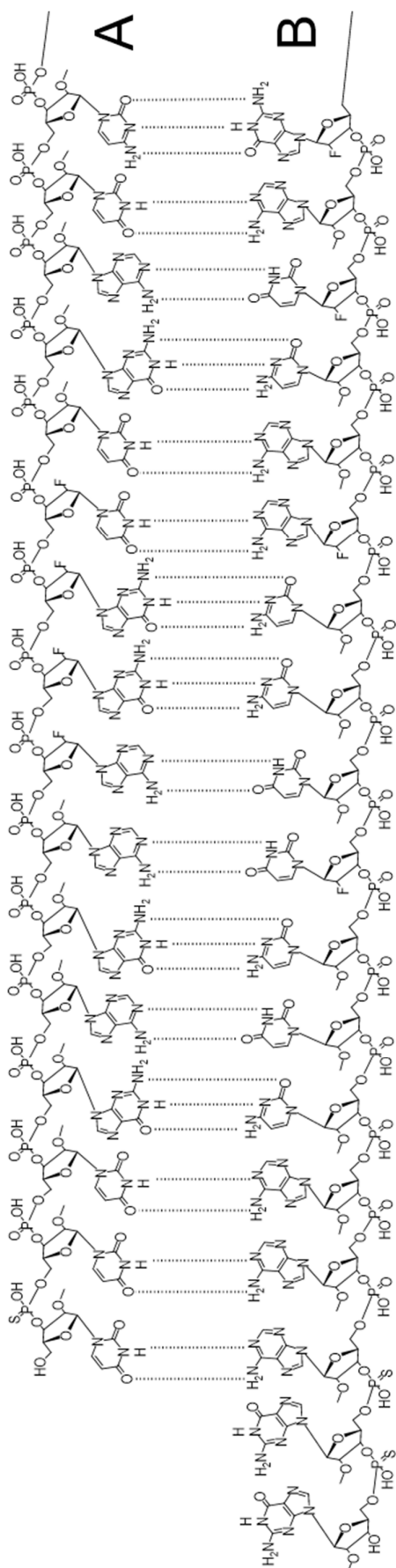


(d) a sense strand comprising SEQ ID NO: 782 and an antisense strand comprising SEQ ID NO: 827, the antisense strand comprising a region of complementarity to a KHK RNA transcript, e.g. KHK mRNA, wherein said dsRNAi is in the form of a conjugate having the structure of:



5

Sense (Passenger)

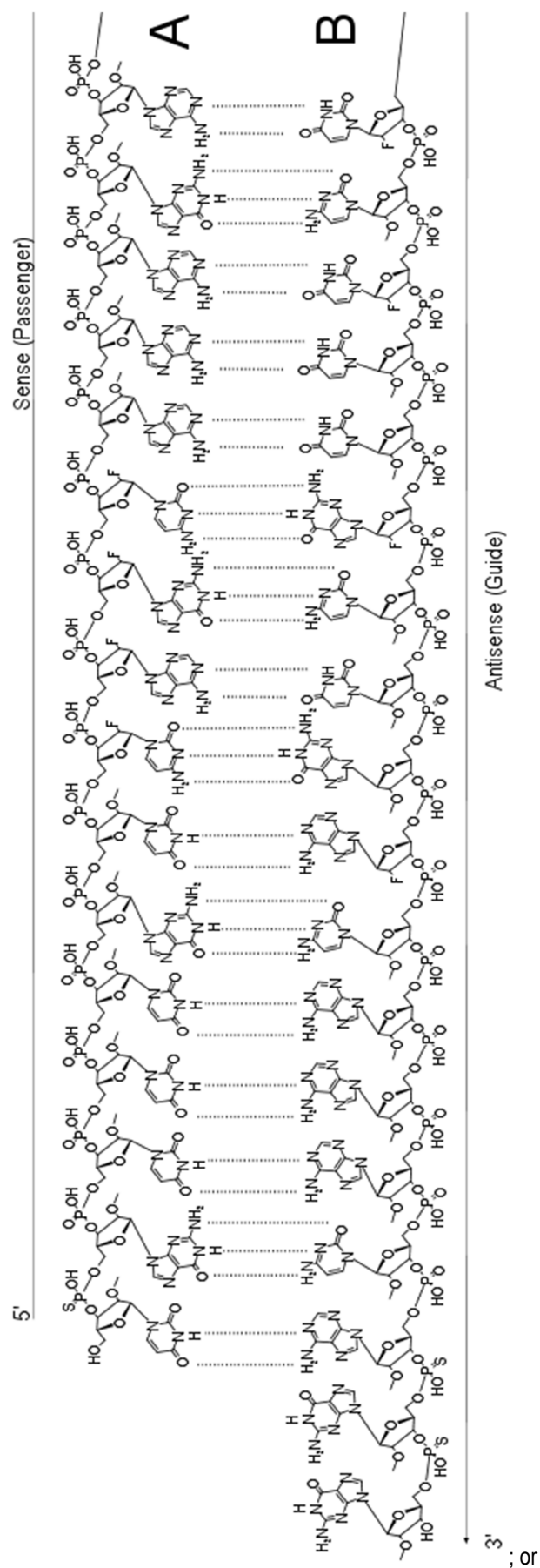


Antisense (Guide)

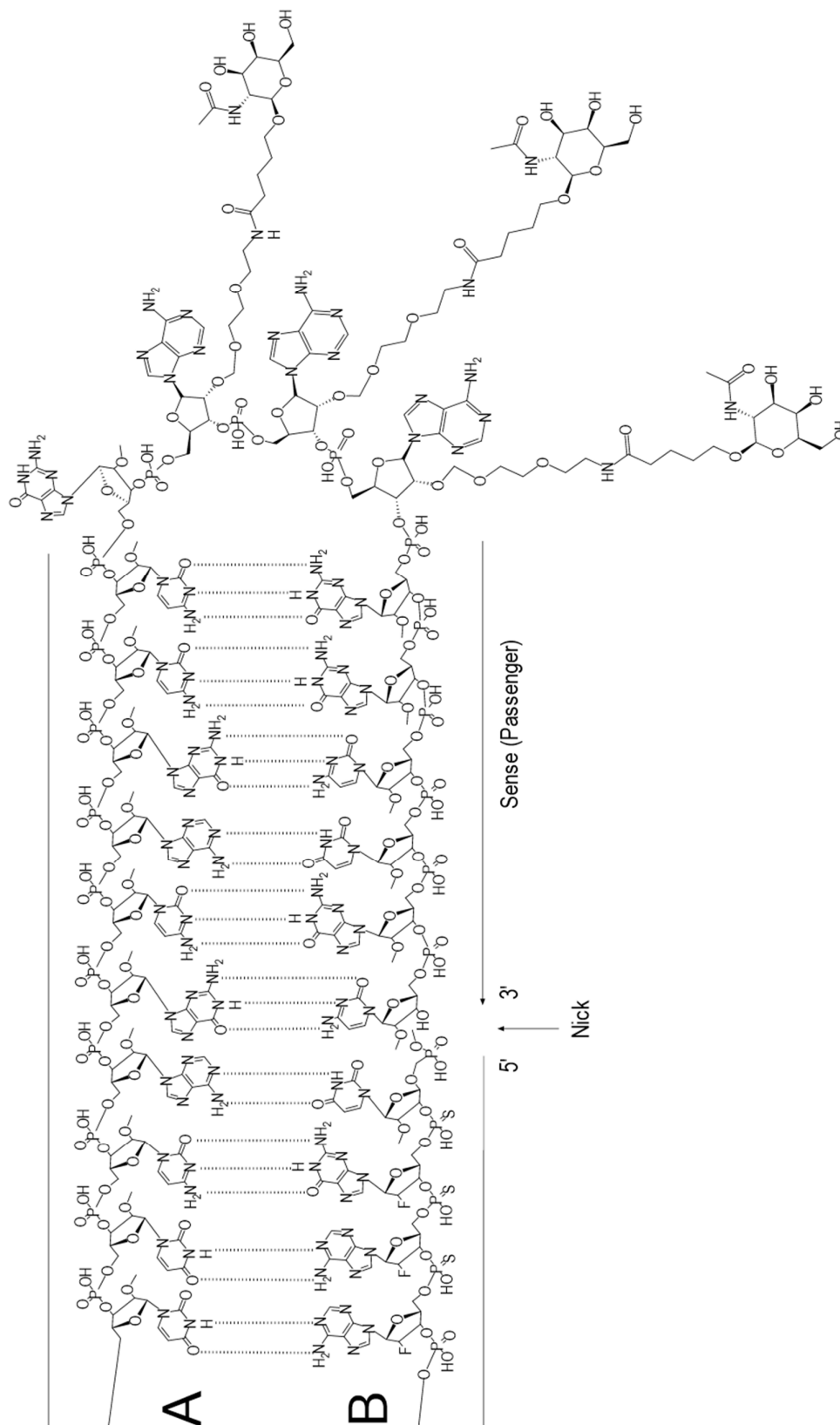
3

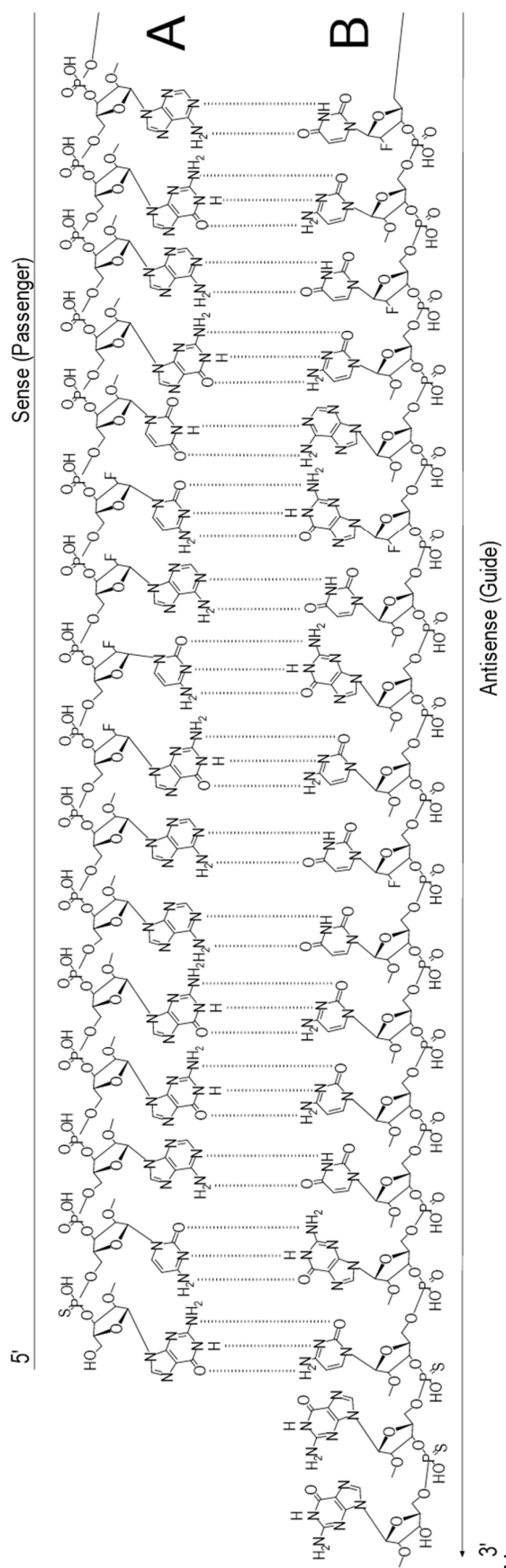
; or

The diagram illustrates a DNA duplex with a nick and a passenger strand. The duplex is oriented with the 5' end on the left and the 3' end on the right. A 'Nick' is indicated on the bottom strand (labeled 'B') between the 5' and 3' ends. A 'Sense (Passenger)' strand is shown extending from the 3' end of the duplex. The chemical structures of the nucleotides, including the sugar-phosphate backbone and the base pairing (A-T, G-C), are detailed. The passenger strand is a single-stranded DNA molecule that is complementary to the 3' end of the duplex.



(f) a sense strand comprising SEQ ID NO: 804 and an antisense strand comprising SEQ ID NO: 849, the antisense strand comprising a region of complementarity to a KHK RNA transcript, e.g. KHK mRNA, wherein said dsRNAi is in the form of a conjugate having the structure of:





or a pharmaceutically acceptable salt thereof.

16. A pharmaceutical composition comprising the dsRNAi oligonucleotide of any one of claims 1-15, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable carrier, delivery agent or excipient.

5

17. The RNAi oligonucleotide of any one of claims 1-15, or the pharmaceutical composition of claim 16, for use as a medicament.

18. The RNAi oligonucleotide of any one of claims 1-15, or the pharmaceutical composition of claim 16, for use in the treatment of a disease, disorder or condition associated with *KHK* expression, optionally for the treatment of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH).

19. The RNAi oligonucleotide of any one of claims 1-15, or the pharmaceutical composition of claim 16, for use according to claim 18, wherein the RNAi oligonucleotide, or pharmaceutical composition, is to be administered in combination with a second composition or therapeutic agent.

15

20. Use of the RNAi oligonucleotide of any one of claims 1-15, or the pharmaceutical composition of claim 16, in the manufacture of a medicament for the treatment of a disease, disorder or condition associated with *KHK* expression, optionally for the treatment of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH).

20

21. The use according to claim 20, wherein the RNAi oligonucleotide, or pharmaceutical composition, is to be administered in combination with a second composition or therapeutic agent.

~~20~~22. An in vitro method for reducing *KHK* expression in a cell, or a population of cells ~~or a subject~~, the method comprising the step of:

25

~~_____i._____~~ contacting the cell or the population of cells with the RNAi oligonucleotide, or a pharmaceutically acceptable salt thereof, of any one of claims 1-~~16~~15, or the pharmaceutical composition of claim ~~17~~16; ~~or~~

~~_____ii._____~~ administering to the subject the RNAi oligonucleotide, or a pharmaceutically acceptable salt thereof of any one of claims 1-16, or the pharmaceutical composition of claim 17.

30