Preparation of Building Blocks
Synthesize Building Blocks:
Antisense
Intact Sense
5'-SplitSense
3'-SplitSense
Ligand
NH2-5'-Sense-3'-SSR
RSS-5'-Se-3'
5'-nse-3'-SSR
(Lig)
Derivatize Building Blocks:
Ligate Ligand to Intact Sense
Derivatize Sense
Derivatize 5'-SplitSense
Derivatize 3'-SplitSense
NH2-5'-Sense-3'-SSR
DBCO-5'-Sense-3'-SSR
(Lig)-5'-Sense-3'-SSR
(Lig)-5'-Sense-3'-SH
NH2-5'-Sense-3'-SH
NH2-5'-Sense-3'-Mal
RSS-5'-Se-3'
HS-5'-Se-3'
Mal-5'-Se-3'
5'-nse-3'-SSR
5'-nse-3'-SH
5'-nse-3'-Mal

FIG. 1

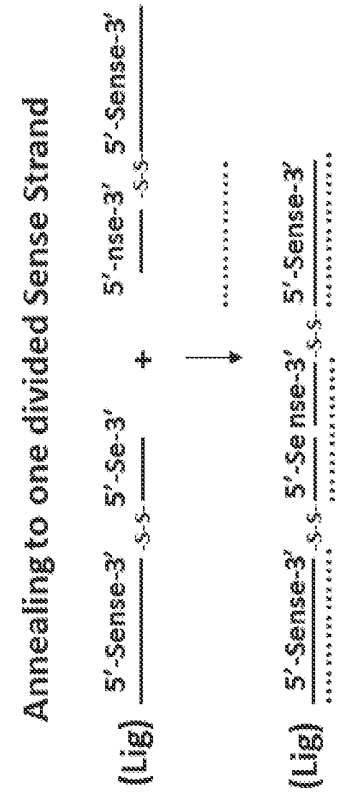
Annealing to one divided Sense Strand
(Lig)
5'-Sense-3'
5'-Se-3'
-S-S-
+
5'-nse-3'
5'-Sense-3'
-S-S-
(Lig)
5'-Sense-3'
-S-S-
5'-Se nse-3'
-S-S-
5'-Sense-3'

FIG. 2

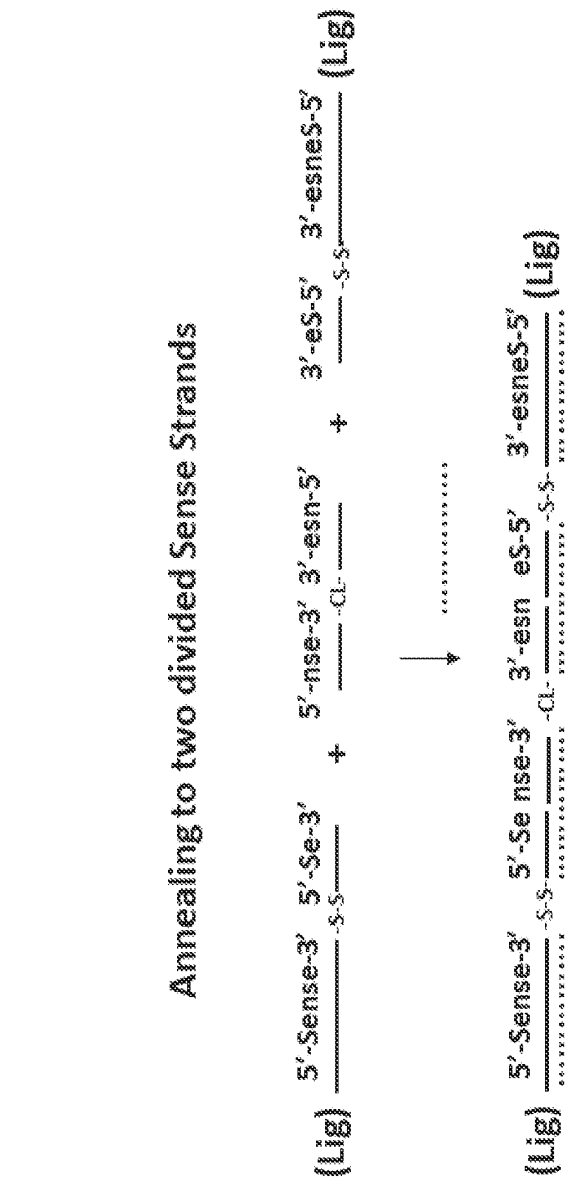
Annealing to two divided Sense Strands
(Lig) 5'-Sense-3' 5'-Se-3' -S-S- + 5'-nse-3' 3'-esn-5' -CL- + 3'-eS-5' 3'-esneS-5' -S-S- (Lig)
(Lig) 5'-Sense-3' -S-S- 5'-Se nse-3' -CL- 3'-esn eS-5' -S-S- 3'-esneS-5' (Lig)

FIG. 3

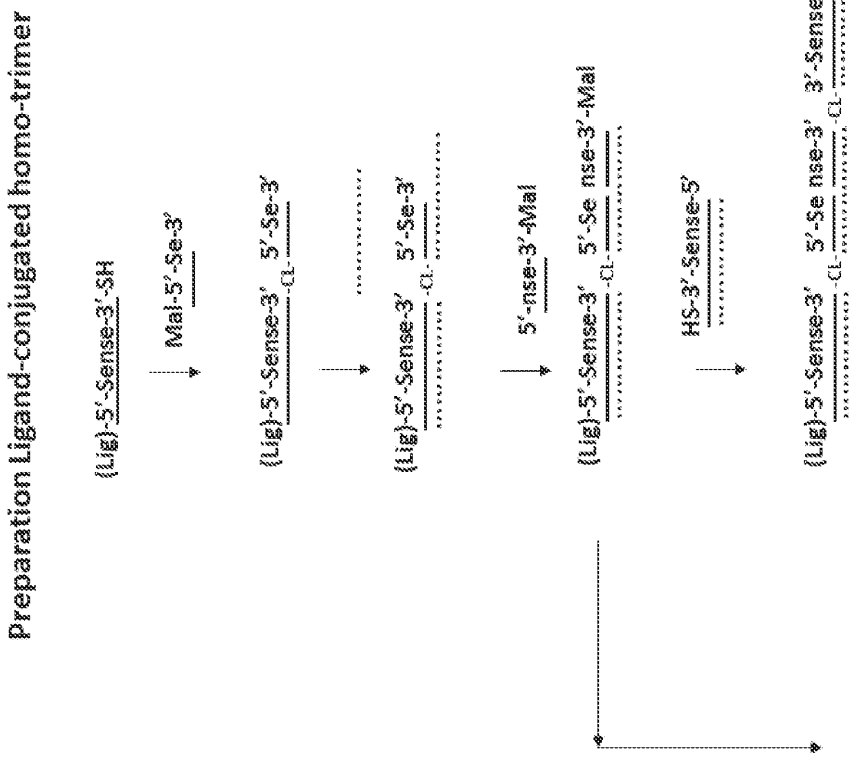
Preparation Ligand-conjugated homo-trimer
(Lig)-5'-Sense-3'-SH
Mal-5'-Se-3'
(Lig)-5'-Sense-3' 5'-Se-3'
Cl
(Lig)-5'-Sense-3' 5'-Se-3'
Cl
5'-nse-3'-Mal
(Lig)-5'-Sense-3' 5'-Se nse-3'-Mal
Cl
HS-3'-Sense-5'
(Lig)-5'-Sense-3' 5'-Se nse-3' 3'-Sense-5'
Cl Cl
To Pentamer

FIG. 4

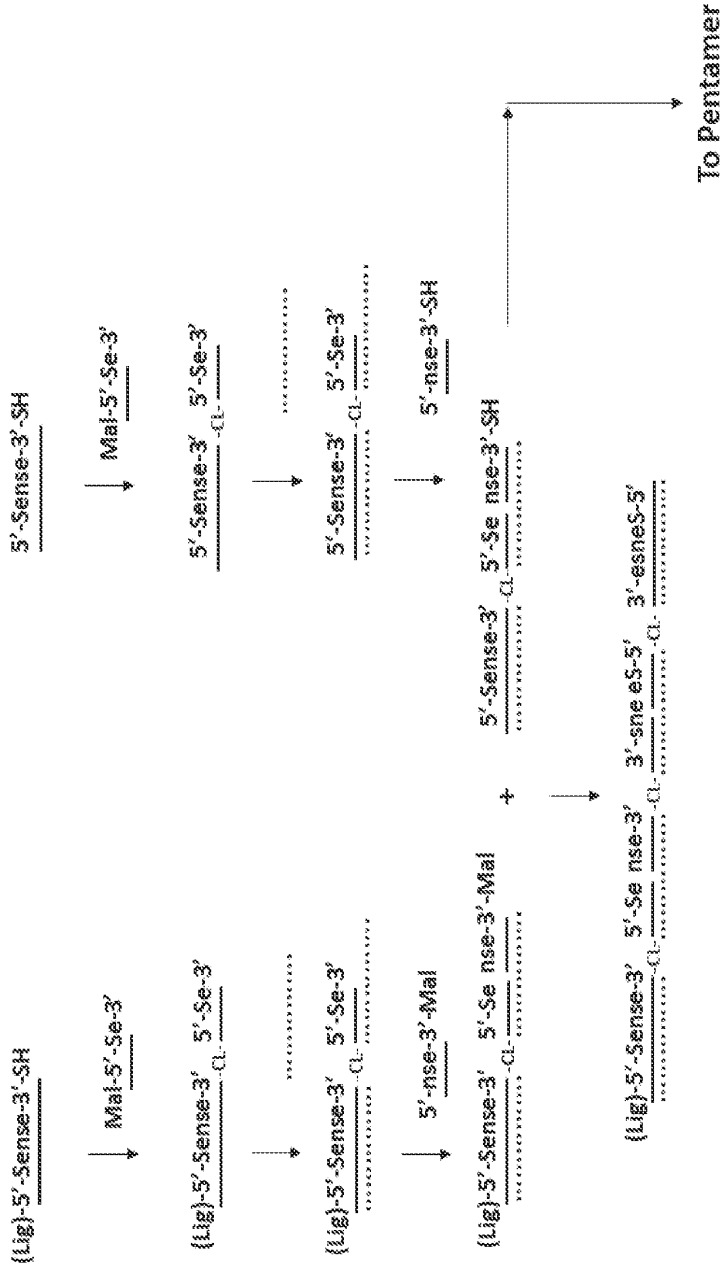
Preparation Ligand-conjugated tetramer
(Lig)-5'-Sense-3'-SH
5'-Sense-3'-SH
Mal-5'-Se-3'
Mal-5'-Se-3'
(Lig)-5'-Sense-3' 5'-Se-3'
5'-Sense-3' 5'-Se-3'
(Lig)-5'-Sense-3' 5'-Se-3'
5'-Sense-3' 5'-Se-3'
5'-nse-3'-Mal
5'-nse-3'-SH
(Lig)-5'-Sense-3' 5'-Se nse-3'-Mal
5'-Sense-3' 5'-Se nse-3'-SH
(Lig)-5'-Sense-3' 5'-Se nse-3' 3'-sne eS-5' 3'-eseS-5'
To Pentamer

FIG. 5

Preparation Ligand-conjugated homo-pentamer
Ex Trimer
(Lig)-5'-Sense-3'
5'-Se
nse-3'-Mal
HS-5'-Se-3'
(Lig)-5'-Sense-3'
5'-Se
nse-3'
5'-Se-3'
5'-nse-3'-Mal
(Lig)-5'-Sense-3'
5'-Se
nse-3'
5'-Se
nse-3'-Mal
+
5'-Sense-3'
5'-Se
nse-3'-SH
Ex Tetramer
(Lig)-5'-Sense-3'
5'-Se
nse-3'
5'-Se
nse-3'
3'-Se
nse-5'
3'-Sense-5'

FIG. 6

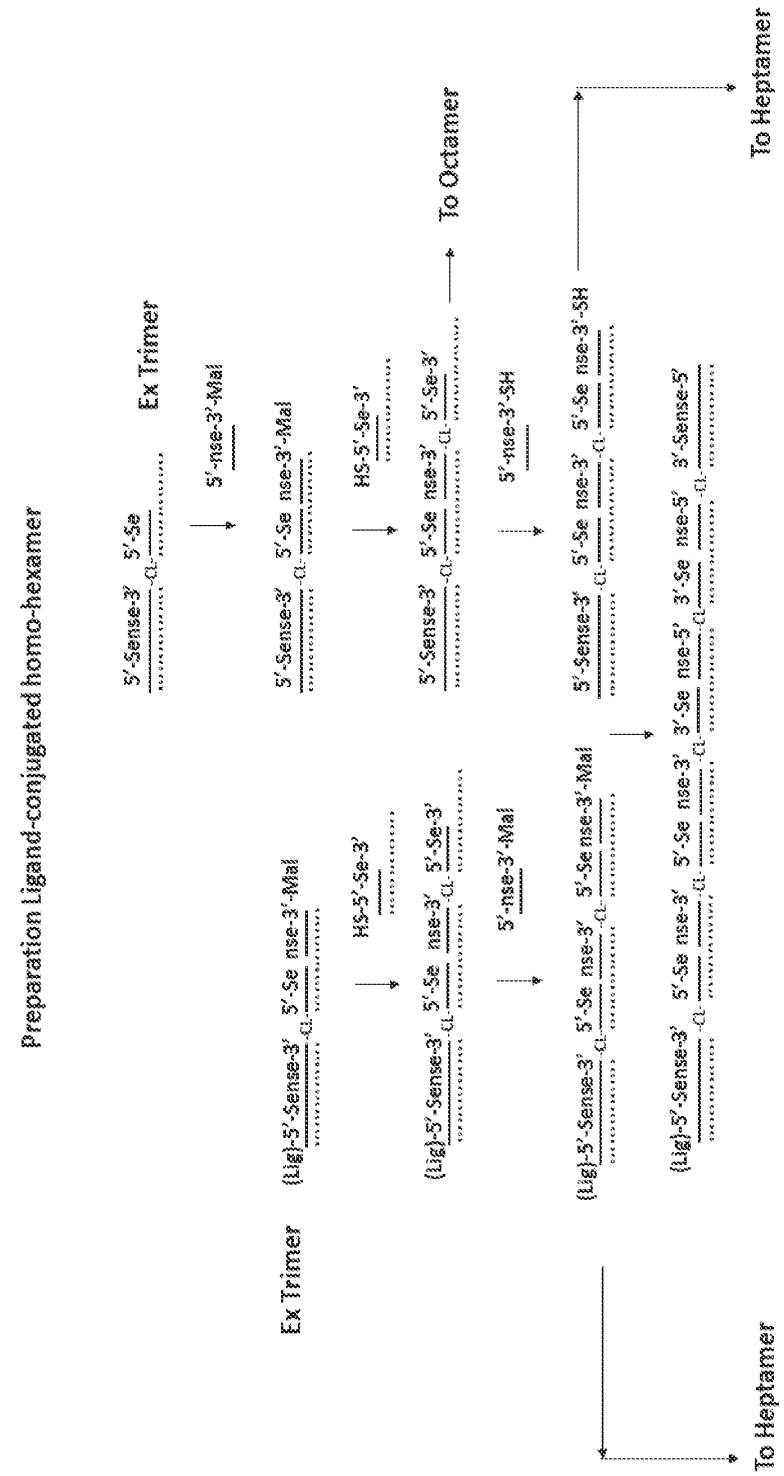
Preparation Ligand-conjugated homo-hexamer
Ex Trimer
5'-Sense-3' 5'-Se
Ex Trimer
5'-nse-3'-Mal
5'-Sense-3' 5'-Se nse-3'-Mal
HS-5'-Se-3'
(Lig)-5'-Sense-3' 5'-Se nse-3' 5'-Se-3'
To Octamer
5'-nse-3'-SH
(Lig)-5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3'-Mal
5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3'-SH
To Heptamer
(Lig)-5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3' 3'-Se nse-5' 3'-Se nse-5' 3'-Sense-5'
To Heptamer

FIG. 7

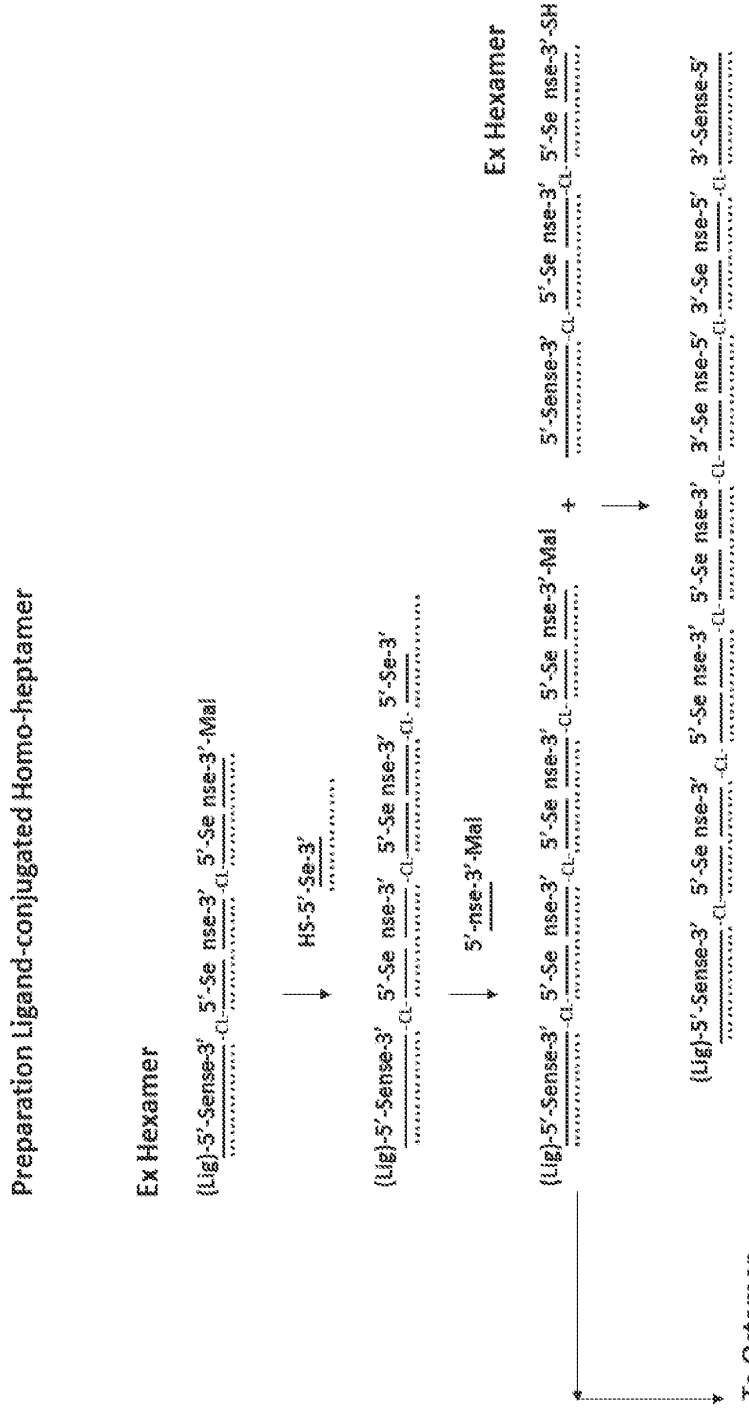
Preparation Ligand-conjugated Homo-heptamer
Ex Hexamer
(Lig)-5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3'-Mal
HS-5'-Se-3'
(Lig)-5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3' 5'-Se-3'
5'-nse-3'-Mal
(Lig)-5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3' 5'-Se nse-3'-Mal
Ex Hexamer
5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3'-SH
(Lig)-5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3' 5'-Se nse-3' 5'-Se nse-5' 3'-Se nse-5' 3'-Sense-5'
To Octamer

FIG. 8

Preparation Ligand-conjugated homo-octamer
Ex Hexamer
5'-Sense-3' 5'-Se nse-3' 5'-Se-3'
5'-nse-3'-Mal
5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3'-Mal
HS-5'-Se-3'
5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3' 5'-Se-3'
5'-nse-3'-SH
Ex Heptamer
(Lig)-5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3'-Mal
5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3' 5'-Se nse-3'-SH
(Lig)-5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3' 5'-Se nse-3' 3'-Se nse-5' 3'-Se nse-5' 3'-Se nse-5' 3'-Sense-5'

FIG. 9

Preparation Ligand-conjugated hetero-pentamer
Ex Trimer
(Lig)-5'-Sense-3' 5'-Se nse-3'-Mal
HS-5'-Se-3'
(Lig)-5'-Sense-3' 5'-Se nse-3' 5'-Se-3'
5'-nse-3'-Mal
(Lig)-5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3'-Mal
(Lig)-5'-Sense-3' 5'-Se nse-3' 5'-Se nse-3' 3'-Se nse-5' 3'-REHTO-5'
5'-OTHER-3'-SH
Mal-5'-Se-3'
5'-OTHER-3' 5'-Se-3'
5'-Sense-3' 5'-Se-3'
5'-nse-3'-SH
5'-OTHER-3' 5'-Se nse-3'-SH
+

FIG. 10

Preparation Ligand-conjugated homo-trimer of siRNA targeting Human DMPK
(Lig)-5'-DMPK-3'-SH
Mal-5'-DM-3'
(Lig)-5'-DMPK-3' 5'-DM-3'
CL
****
(Lig)-5'-DMPK-3' 5'-DM-3'
CL
********** **********
5'-PK-3'-Mal
(Lig)-5'-DMPK-3' 5'-DM PK-3'-Mal
CL
********** **********
HS-3'-KPMD-5'
****
(Lig)-5'-DMPK-3' 5'-DMPK-3' 3'-KPMD-5'
CL CL
********** ********** **********

FIG. 11

Preparation Ligand-conjugated homo-trimer of siRNA targeting Human Huntingtin
(Lig)-5'-HTTs-3'-SH
Mal-5'-HT-3'
(Lig)-5'-HTTs-3' -CL- 5'-HT-3'
(Lig)-5'-HTTs-3' -CL- 5'-HT-3'
5'-Ts-3'-Mal
(Lig)-5'-HHTs-3' -CL- 5'-HT Ts-3'-Mal
HS-3'-sTTH-5'
(Lig)-5'-HTTs-3' -CL- 5'-HT Ts-3' -CL- 3'-sTTH-5'

FIG. 12