

CLAIMS

What is claimed is:

1. A conjugate comprising a modified antisense oligonucleotide and a cell-penetrating peptide, wherein:
 - the modified antisense oligonucleotide is 18-40 subunits in length, comprising a targeting sequence complementary to a target region within intron 1 (SEQ ID NO: 1) of a pre-mRNA of human acid alpha-glucosidase (GAA) gene, wherein
 - the antisense oligonucleotide comprises a morpholino oligomer;
 - the antisense oligonucleotide is covalently linked to the cell-penetrating peptide;
 - each subunit of the antisense oligonucleotide comprises a nucleobase or is an abasic subunit, wherein each subunit is taken together in order from the 5' end of the antisense oligonucleotide to the 3' end of the antisense oligonucleotide form the targeting sequence;
 - at least one subunit is an abasic subunit; and
 - wherein the targeting sequence, except for the abasic subunit or subunits, is at least 80% complementary to the target region.
2. The conjugate of claim 1, wherein the target region comprises a sequence selected from the group consisting of SEQ ID NO: 2 (GAA-IVS1(-189-167)) and SEQ ID NO: 3 (GAA-IVS1(-80-24)).
3. The conjugate of claim 2, wherein the target region comprises the sequence set forth as SEQ ID NO: 2.
4. The conjugate of claim 2, wherein the target region comprises the sequence set forth as SEQ ID NO: 3.
5. The conjugate of claim 1 or 2, wherein the target region is selected from GAA-IVS1(-189-167), GAA-IVS1(-80-56), GAA-IVS1(-76-52), GAA-IVS1(-74-55), GAA-IVS1(-72-48), GAA-IVS1(-71-47), GAA-IVS1(-70-46), GAA-IVS1(-69-45), GAA-IVS1(-66-42), GAA-IVS1(-65-41), and GAA-IVS1(-49-24).
6. The conjugate of claim 1 or 5, wherein the target region is GAA-IVS1(-189-167).

7. The conjugate of claim 1 or 6, wherein the targeting sequence comprises the sequence CCA GAA GGA AXX XCG AGA AAA GC (SEQ ID NO: 4), wherein each X is independently selected from guanine (G) or is abasic (B), wherein at least one X is B.
8. The conjugate of claim 1 or 7, wherein the targeting sequence comprises a sequence selected from the group consisting of:
 - i) SEQ ID NO: 5 (CCA GAA GGA AGG BCG AGA AAA GC);
 - ii) SEQ ID NO: 6 (CCA GAA GGA AGB GCG AGA AAA GC);
 - iii) SEQ ID NO: 7 (CCA GAA GGA ABG GCG AGA AAA GC);
 - iv) SEQ ID NO: 8 (CCA GAA GGA AGB BCG AGA AAA GC);
 - v) SEQ ID NO: 9 (CCA GAA GGA ABB GCG AGA AAA GC); and
 - vi) SEQ ID NO: 10 (CCA GAA GGA ABG BCG AGA AAA GC).
10. The conjugate of claim 1 or 5, wherein the target region is selected from the group consisting of GAA-IVS1(-80-56), GAA-IVS1(-76-52), GAA-IVS1(-74-55), GAA-IVS1(-72-48), GAA-IVS1(-71-47), GAA-IVS1(-70-46), GAA-IVS1(-69-45), GAA-IVS1(-66-42), GAA-IVS1(-65-41), and GAA-IVS1(-49-24).
11. The conjugate of claim 1 or 10, wherein the target region is selected from the group consisting of GAA-IVS1(-72-48), GAA-IVS1(-71-47), GAA-IVS1(-70-46), GAA-IVS1(-69-45), GAA-IVS1(-66-42), and GAA-IVS1(-65-41).
12. The conjugate of claim 1 or 11, wherein the targeting sequence comprises a sequence selected from the group consisting of:
 - i) SEQ ID NO: 11 (CTC ACX XXX CTC TCA AAG CAG CTC T);
 - ii) SEQ ID NO: 12 (ACT CAC XXX XCT CTC AAA GCA GCT C);
 - iii) SEQ ID NO: 13 (CAC TCA CXX XXC TCT CAA AGC AGC T);
 - iv) SEQ ID NO: 14 (GCA CTC ACX XXX CTC TCA AAG CAG C);
 - v) SEQ ID NO: 15 (GCG GCA CTC ACX XXX CTC TCA AAG C);
 - vi) SEQ ID NO: 16 (GGC GGC ACT CAC XXX XCT CTC AAA G);
 wherein each X is independently selected from guanine (G) or is abasic (B), wherein at least one X is B.
13. The conjugate of claim 1 or 12, wherein the targeting sequence is selected from the group consisting of:
 - i) SEQ ID NO: 17 (GCA CTC ACB GGG CTC TCA AAG CAG C);
 - ii) SEQ ID NO: 18 (GCA CTC ACG BGG CTC TCA AAG CAG C);

- iii) SEQ ID NO: 19 (GCA CTC ACG GBG CTC TCA AAG CAG C);
- iv) SEQ ID NO: 20 (GCA CTC ACG GGB CTC TCA AAG CAG C);
- v) SEQ ID NO: 21 (GCA CTC ACB BGG CTC TCA AAG CAG C);
- vi) SEQ ID NO: 22 (GCA CTC ACG BBG CTC TCA AAG CAG C);
- vii) SEQ ID NO: 23 (GCA CTC ACG GBB CTC TCA AAG CAG C); and
- viii) SEQ ID NO: 24 (GGC GGC ACT CAC GBB GCT CTC AAA G).

14. The conjugate of any one of claims 1 to 13, wherein the targeting sequence, except for the abasic subunit or subunits, is at least 84%, at least 88%, or at least 92% complementary to the target region.

15. The conjugate of any one of claims 1 to 13, wherein the targeting sequence, except for the abasic subunit or subunits, is at least 90% complementary to the target region.

16. The conjugate of any one of claims 1 to 5, wherein the targeting sequence, except for the abasic subunit or subunits, is at least 95% complementary to the target region.

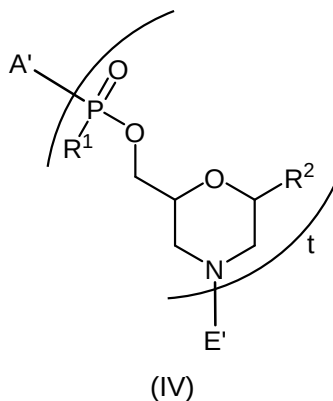
17. The conjugate of any one of claims 1 to 5, wherein the targeting sequence, except for the abasic subunit or subunits, is 100% complementary to the target region.

18. The conjugate of any one of claims 1 to 4, wherein each abasic subunit is at least 8 subunits from the 5' or 3' end of the targeting sequence.

19. The conjugate of any one of claims 1 to 4, wherein the antisense oligonucleotide comprises 1 to 5 abasic subunits.

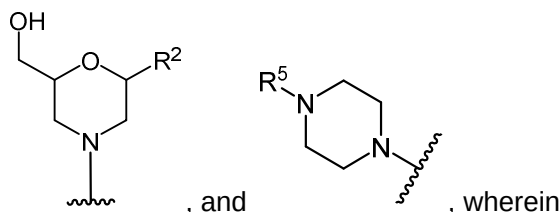
20. The conjugate of any one of claims 1 to 4 or 19, wherein the antisense oligonucleotide comprises 1, 2, 3, or 4 abasic subunits.

21. The conjugate of any one of claims 1 to 20, wherein the conjugate is a compound of Formula IV:



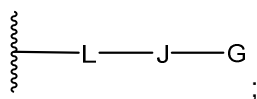
or a pharmaceutically acceptable salt thereof,
wherein:

A' is selected from $-N(H)CH_2C(O)NH_2$, $-N(C_{1-6}\text{-alkyl})CH_2C(O)NH_2$,



R⁵ is $-C(O)(O\text{-alkyl})_x\text{-OH}$, wherein x is 3-10 and each alkyl group is, independently at each occurrence, C₂₋₆-alkyl,

or R⁵ is selected from H, $-C(O)C_{1-6}\text{-alkyl}$, trityl, monomethoxytrityl, $-(C_{1-6}\text{-alkyl})\text{-R}^6$, $-(C_{1-6}\text{-heteroalkyl})\text{-R}^6$, aryl-R⁶, heteroaryl-R⁶, $-C(O)O\text{-}(C_{1-6}\text{-alkyl})\text{-R}^6$, $-C(O)O\text{-aryl-R}^6$, $-C(O)O\text{-heteroaryl-R}^6$, and



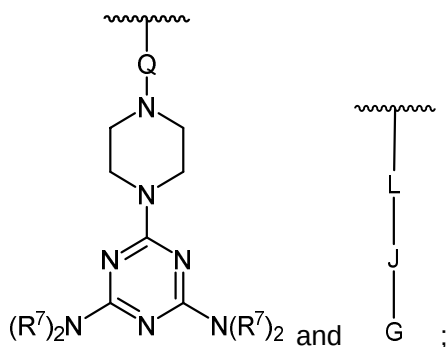
R⁶ is selected from OH, SH, and NH₂, or R⁶ is O, S, or NH, each of which is covalently linked to a solid support;

each R¹ is independently selected from OH and $-N(R^3)(R^4)$, wherein each R³ and R⁴ are, independently at each occurrence, H or $-C_{1-6}\text{-alkyl}$;

each R² is independently, at each occurrence, selected from H (abasic), a nucleobase, and a nucleobase functionalized with a chemical protecting group, wherein the nucleobase, independently at each occurrence, comprises a C₃₋₆-heterocyclic ring selected from pyridine, pyrimidine, purine, and deaza-purine;

t is 8-40;

E' is selected from H, $-C_{1-6}\text{-alkyl}$, $-C(O)C_{1-6}\text{-alkyl}$, benzoyl, stearoyl, trityl, monomethoxytrityl, dimethoxytrityl, trimethoxytrityl,



wherein

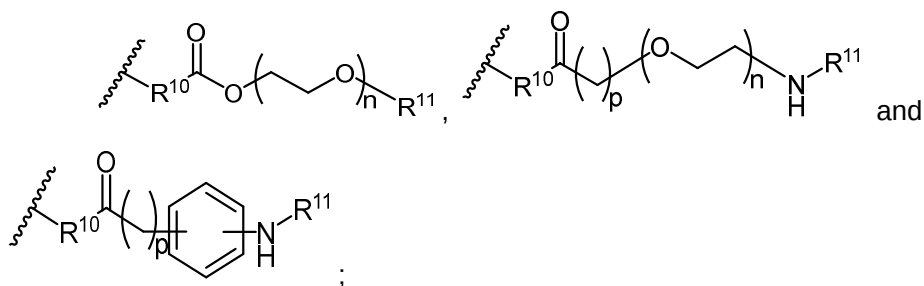
Q is -C(O)(CH₂)₆C(O)- or -C(O)(CH₂)₂S₂(CH₂)₂C(O)-;

R⁷ is -(CH₂)₂OC(O)N(R⁸)₂, wherein R⁸ is -(CH₂)₆NHC(=NH)NH₂;

L is selected from glycine, proline, W, W-W, or R⁹, wherein L is covalently linked by an amide bond to the N-terminus or C-terminus of J;

W is -C(O)-(CH₂)_m-NH-, wherein m is 2 to 12;

R⁹ is selected from the group consisting of:

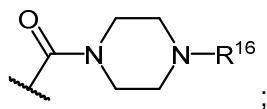


n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

p is 2, 3, 4, or 5;

R¹⁰ is selected from a bond, glycine, proline, W, or W-W;

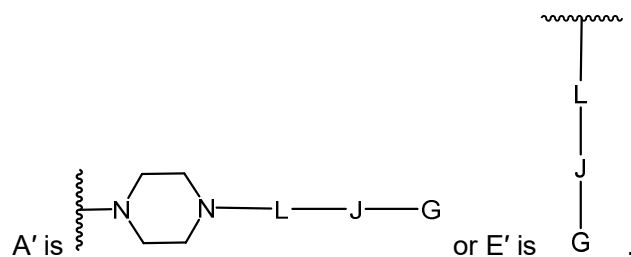
R¹¹ is selected from the group consisting of glycine, proline, W, W-W, and



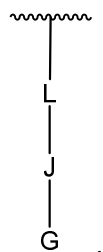
R¹⁶ is selected from a bond, glycine, proline, W, or W-W; wherein R¹⁶ is covalently linked by an amide bond to the N-terminus or C-terminus of J; J is a cell-penetrating peptide; and

G is selected from H, -C(O)C₁₋₆-alkyl, benzoyl, and stearyl, wherein G is covalently linked to J;

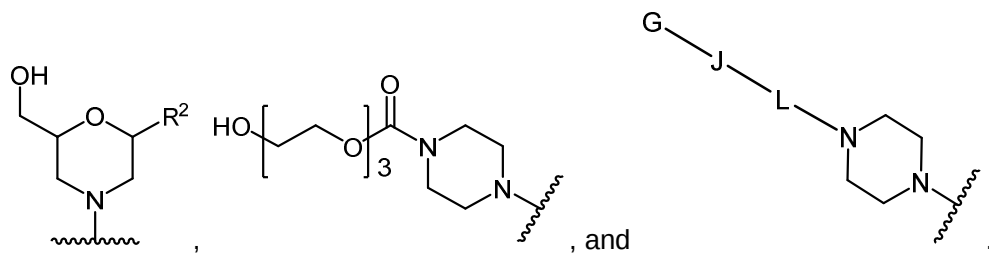
provided that



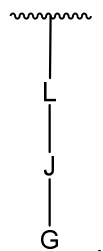
22. The conjugate of claim 21, wherein E' is selected from H, -C₁₋₆-alkyl, -C(O)C₁₋₆-alkyl, benzoyl, stearoyl, trityl, monomethoxytrityl, dimethoxytrityl, trimethoxytrityl, and



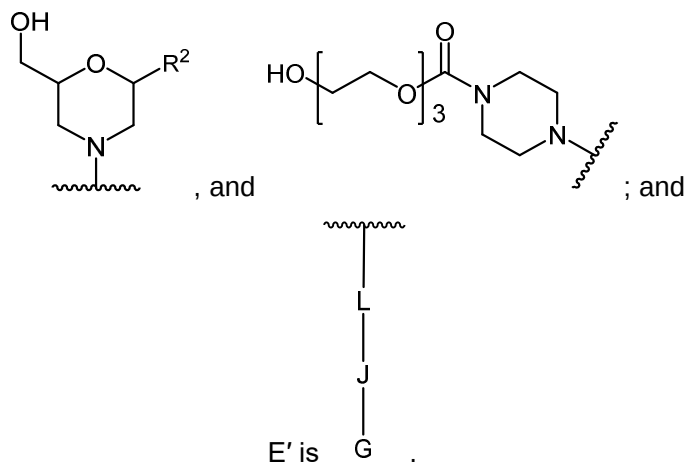
23. The conjugate of claim 21 or 22, wherein A' is selected from -N(C₁₋₆-alkyl)CH₂C(O)NH₂,



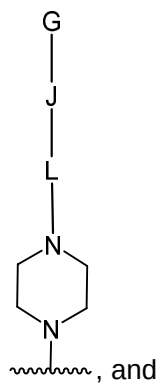
24. The conjugate of any one of claims 21-23, wherein E' is selected from H, -C(O)CH₃, benzoyl, stearoyl, trityl, 4-methoxytrityl, and



25. The conjugate of any one of claims 21-24, wherein A' is selected from -N(C₁₋₆-alkyl)CH₂C(O)NH₂,

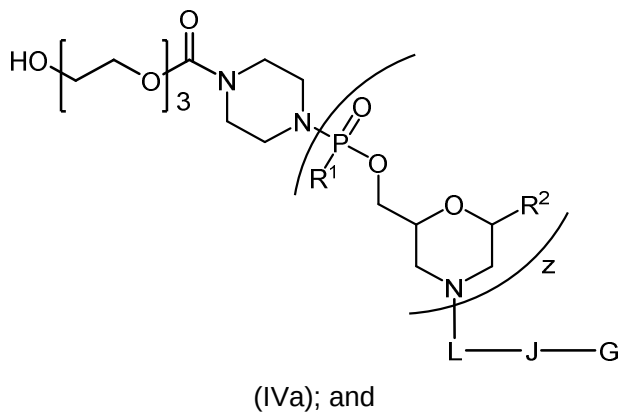


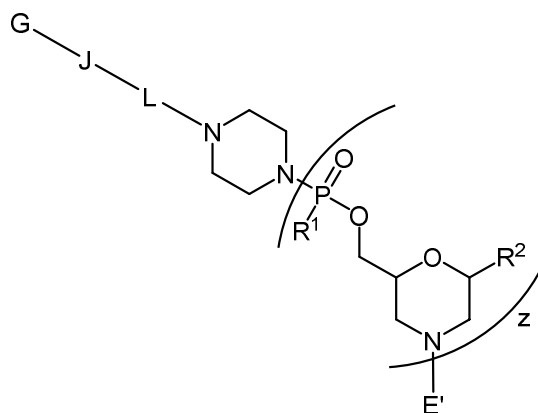
26. The conjugate of any one of claims 21-24, wherein A' is



E' is selected from H, -C(O)CH₃, trityl, 4-methoxytrityl, benzoyl, and stearoyl.

27. The conjugate of claim 21, wherein the peptide-oligonucleotide conjugate of Formula IV is a peptide-oligonucleotide conjugate selected from:





(IVb)

wherein E' is selected from H, C₁₋₆-alkyl, -C(O)CH₃, benzoyl, and stearoyl.

28. The conjugate of any one of claims 21 to 27, wherein the conjugate is of the Formula (IVa).
29. The conjugate of any one of claims 21 to 27, wherein the conjugate is of the Formula (IVb).
30. The conjugate of any one of claims 21 to 27, wherein each R¹ is -N(CH₃)₂.
31. The conjugate of any one of claims 21 to 30, wherein each nucleobase, independently at each occurrence, is selected from adenine, guanine, cytosine, 5-methylcytosine, thymine, uracil, and hypoxanthine.
32. The conjugate of any one of claims 21 to 31, wherein the targeting sequence comprises the sequences:
 - i) SEQ ID NO: 4 (CCA GAA GGA AXX XCG AGA AAA GC);
 - ii) SEQ ID NO: 11 (CTC ACX XXX CTC TCA AAG CAG CTC T);
 - iii) SEQ ID NO: 12 (ACT CAC XXX XCT CTC AAA GCA GCT C);
 - iv) SEQ ID NO: 13 (CAC TCA CXX XXC TCT CAA AGC AGC T);
 - v) SEQ ID NO: 14 (GCA CTC ACX XXX CTC TCA AAG CAG C);
 - vi) SEQ ID NO: 15 (GCG GCA CTC ACX XXX CTC TCA AAG C);
 - vii) SEQ ID NO: 16 (GGC GGC ACT CAC XXX XCT CTC AAA G);

wherein each X is independently selected from guanine (G) or is abasic (B), wherein at least one X is B.

33. The conjugate of any one of claims 21 to 33, wherein the targeting sequence comprises a sequence selected from the group consisting of:

- i) SEQ ID NO: 5 (CCA GAA GGA AGG BCG AGA AAA GC);
- ii) SEQ ID NO: 6 (CCA GAA GGA AGB GCG AGA AAA GC);
- iii) SEQ ID NO: 7 (CCA GAA GGA ABG GCG AGA AAA GC);
- iv) SEQ ID NO: 8 (CCA GAA GGA AGB BCG AGA AAA GC);
- v) SEQ ID NO: 9 (CCA GAA GGA ABB GCG AGA AAA GC);
- vi) SEQ ID NO: 10 (CCA GAA GGA ABG BCG AGA AAA GC);
- vii) SEQ ID NO: 17 (GCA CTC ACB GGG CTC TCA AAG CAG C);
- viii) SEQ ID NO: 18 (GCA CTC ACG BGG CTC TCA AAG CAG C);
- ix) SEQ ID NO: 19 (GCA CTC ACG GBG CTC TCA AAG CAG C);
- x) SEQ ID NO: 20 (GCA CTC ACG GGB CTC TCA AAG CAG C);
- xi) SEQ ID NO: 21 (GCA CTC ACB BGG CTC TCA AAG CAG C);
- xii) SEQ ID NO: 22 (GCA CTC ACG BBG CTC TCA AAG CAG C);
- xiii) SEQ ID NO: 23 (GCA CTC ACG GBB CTC TCA AAG CAG C); and
- xiv) SEQ ID NO: 24 (GGC GGC ACT CAC GBB GCT CTC AAA G).

34. The conjugate of any one of claims 21 to 33, wherein L is glycine.

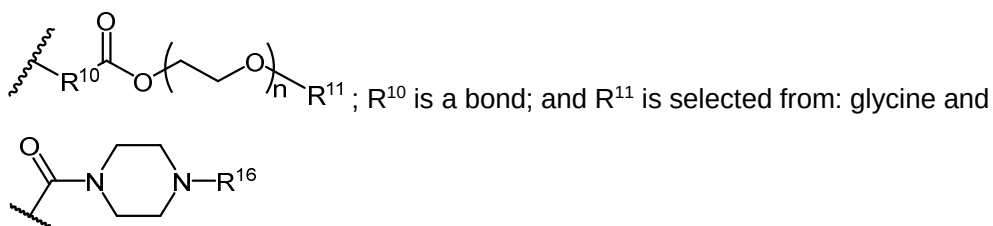
35. The conjugate of any one of claims 21 to 33, wherein L is proline.

36. The conjugate of any one of claims 21 to 33, wherein L is -C(O)-(CH₂)₅-NH-.

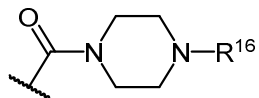
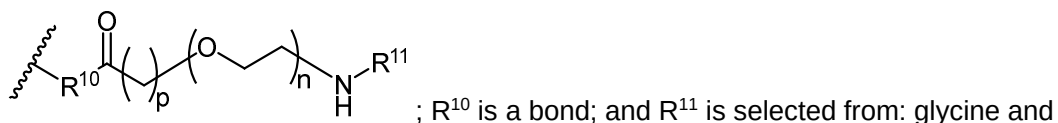
37. The conjugate of any one of claims 21 to 33, wherein L is -C(O)-(CH₂)₂-NH-.

38. The conjugate of any one of claims 21 to 33, wherein L is -C(O)-(CH₂)₂-NH-C(O)-(CH₂)₅-NH-.

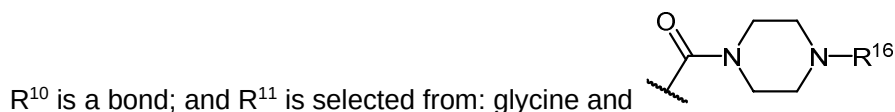
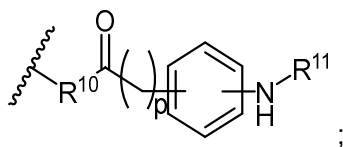
39. The conjugate of any one of claims 21 to 33, wherein L is



40. The conjugate of any one of claims 21 to 33, wherein L is



41. The conjugate of any one of claims 21 to 33, wherein L is



42. The conjugate of any one of claims 21 to 39, wherein J is selected from rTAT, TAT, R_9F_2 , $R_5F_2R_4$, R_4 , R_5 , R_6 , R_7 , R_8 , R_9 , $(RXR)_4$, $(RXR)_5$, $(RXRRBR)_2$, $(RAR)_4F_2$, $(RGR)_4F_2$.

43. The conjugate of any one of claims 21 to 42, wherein G is selected from H, $C(O)CH_3$, benzoyl, and stearoyl.

44. The conjugate of any one of claims 21 to 43, wherein G is H or $-C(O)CH_3$.

45. The conjugate of any one of claims 21 to 44, wherein G is H.

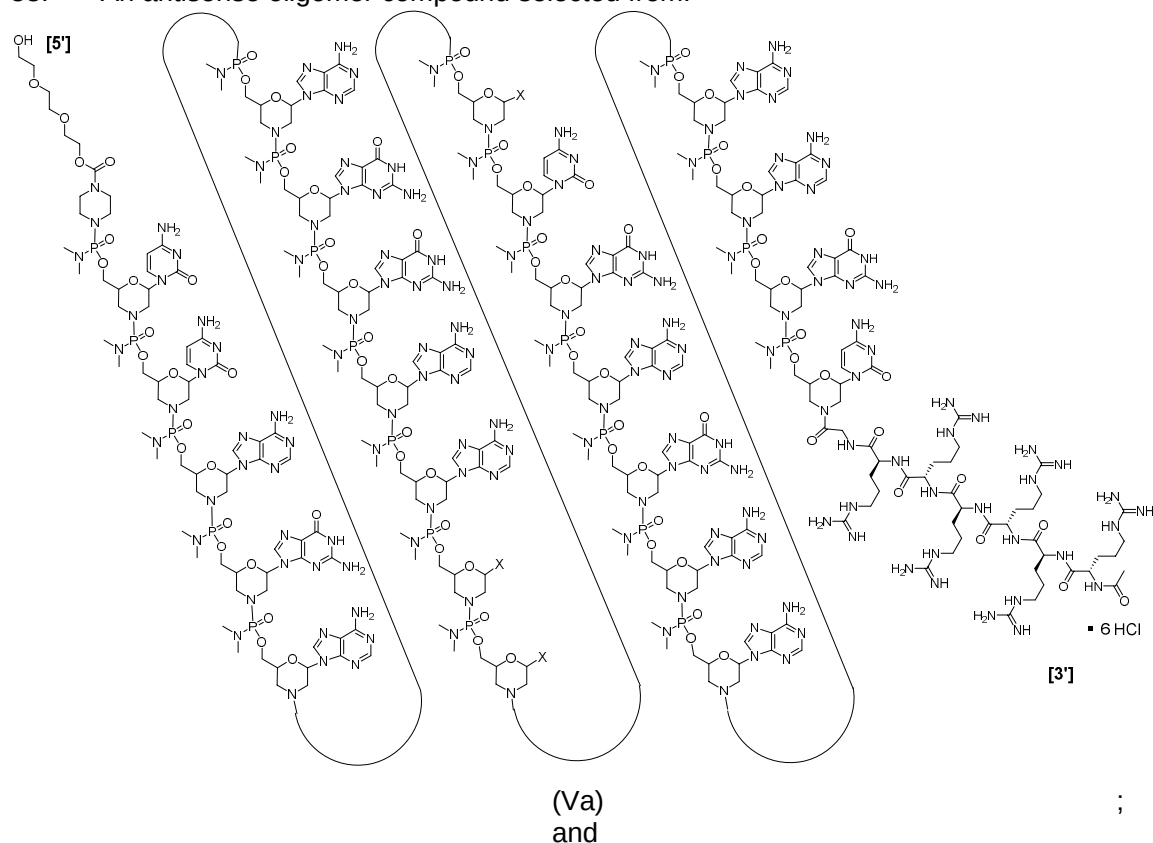
46. The conjugate of any one of claims 21 to 44, wherein G is $-C(O)CH_3$.

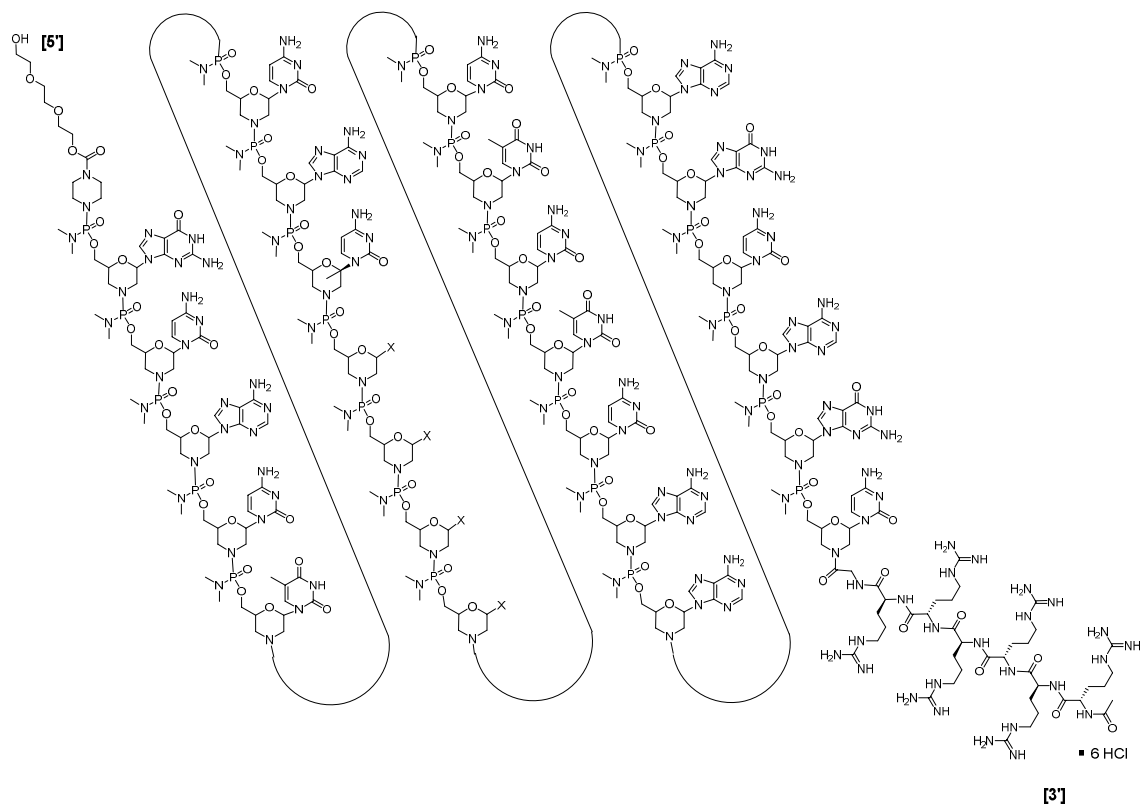
47. A pharmaceutical composition comprising the conjugate of any one of claims 1 to 46, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable carrier.

48. The conjugate of any one of claims 1 to 46 or the pharmaceutical composition of claim 47 for use in therapy.

49. Use of the conjugate of any one of claims 1 to 46 or the pharmaceutical composition of claim 47 in the manufacture of a medicament for the treatment of Pompe disease in a

53. An antisense oligomer compound selected from:

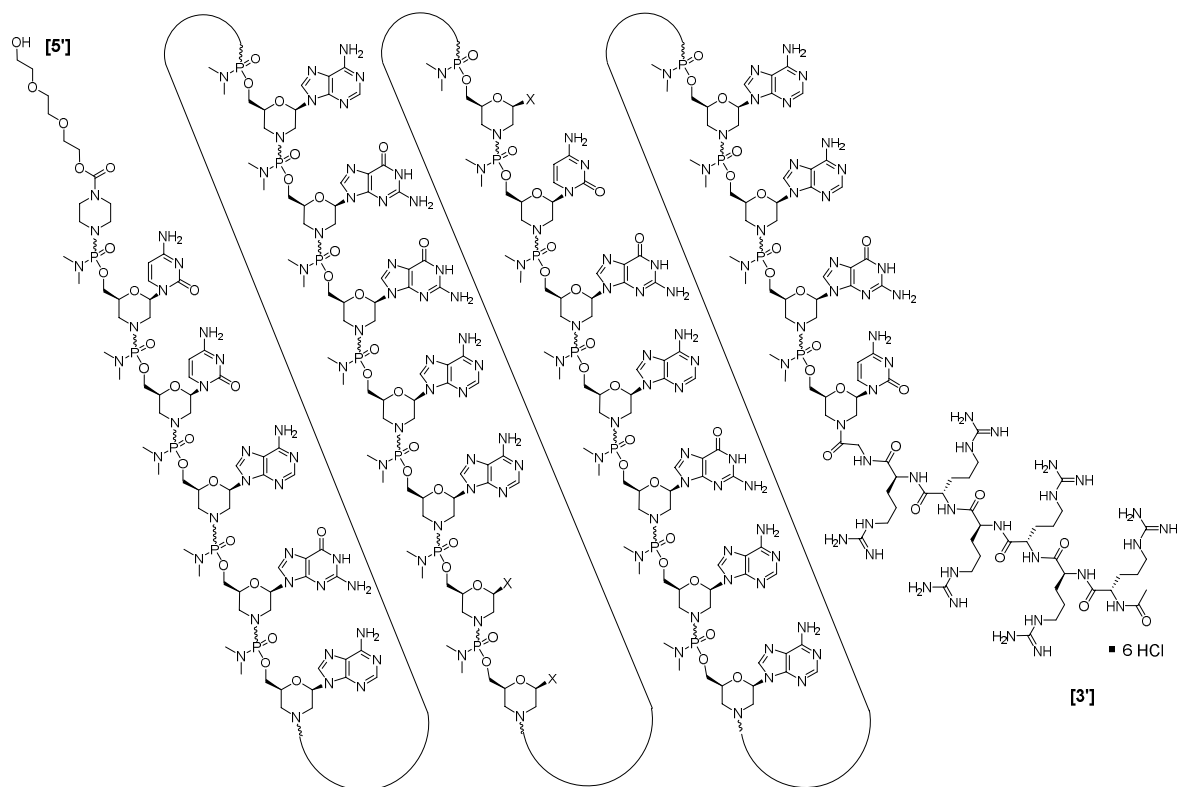




(Vc)

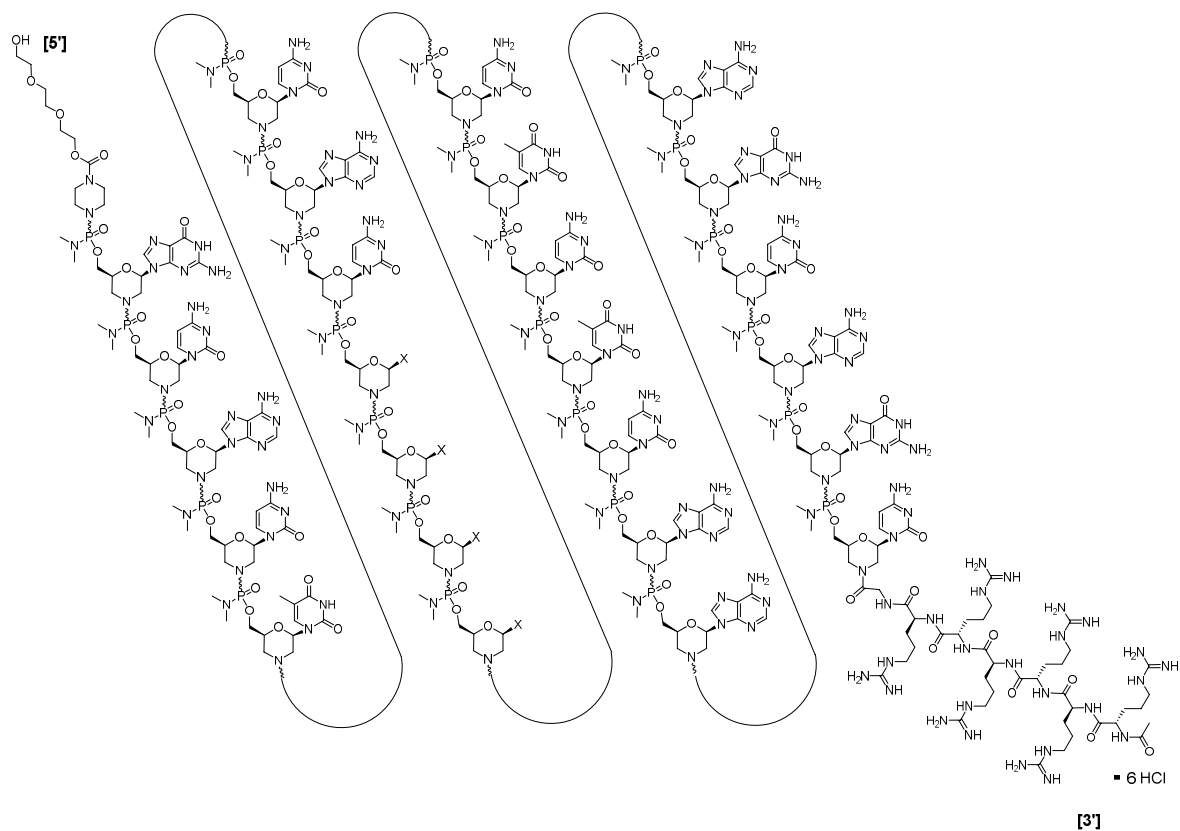
wherein each X is independently selected from guanine (G) or is abasic (B), wherein at least one X is B.

54. The antisense oligomer compound of claim 53, wherein the antisense oligomer compound is:



wherein each X is independently selected from guanine (G) or is abasic (B), wherein at least one X is B.

55. The antisense oligomer compound of claim 53, wherein the antisense oligomer compound is:



wherein each X is independently selected from guanine (G) or is abasic (B), wherein at least one X is B, optionally wherein B is H.