

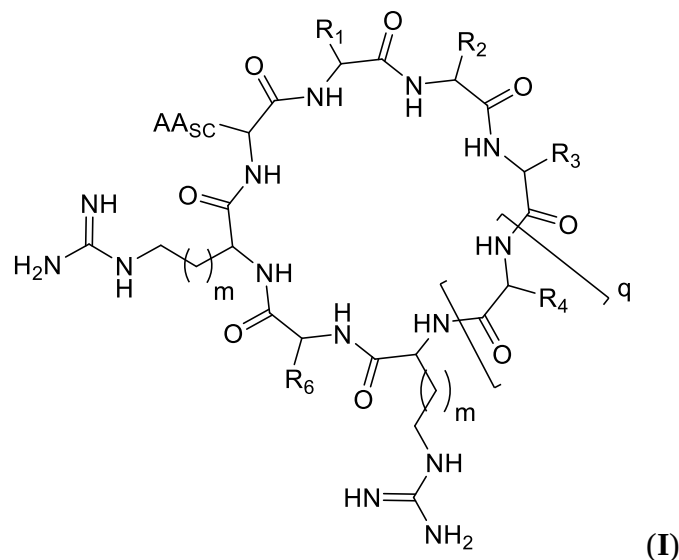
Appelbaum, J S et al. Chem. Biol., 2012, 19, 819-830). Cyclic CPPs (cCPPs) with improved properties have been described for use in intracellular delivery of a cargo moiety (U.S. Patent Publication Nos. 2017/0190743 and 2017/0355730).

[0004] There is still an unmet need for effective compositions and methods for intracellular delivery of a therapeutic agent, particularly in a manner that allows for modulation of the tissue distribution and/or retention of the agent. The compositions and methods disclosed herein address these and other needs.

SUMMARY

[0004a] In a first aspect of the present disclosure, there is provided a compound comprising:

(a) a cyclic cell penetrating peptide (CPP) of Formula (I):



or a protonated form thereof,

wherein:

one of R₁, R₂, and R₃ are H;

two of R₁, R₂, and R₃ are -CH₂Ph;

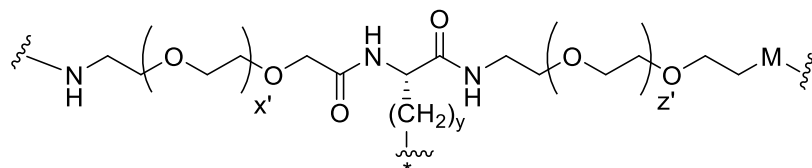
R₄ and R₆ are independently H or an amino acid side chain;

AA_{SC} is an amino acid side chain;

q is 1, 2, 3 or 4; and

each m is independently an integer 0, 1, 2, or 3;

(b) a linker comprising the structure



5

wherein:

x' is an integer from 1-23;

y is an integer from 1-5;

z' is an integer from 1-23;

* is the point of attachment to the AA_{SC}, and

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M is a bonding group;

(c) an exocyclic peptide (EP) comprising from 2 to 10 amino acid residues and

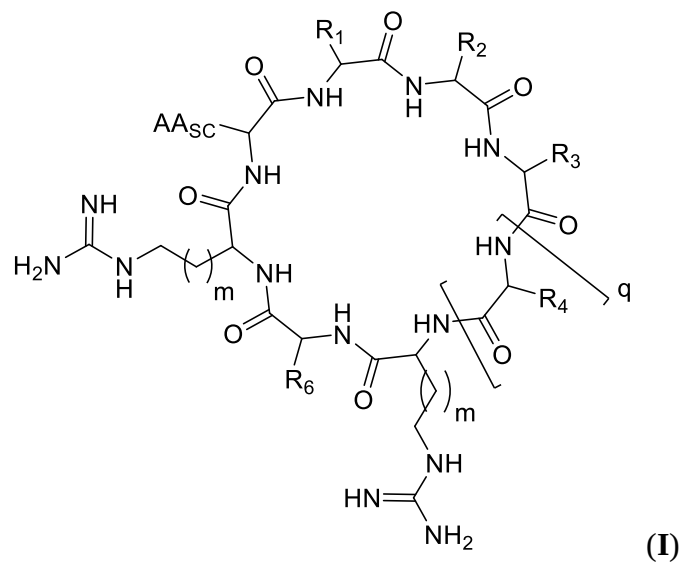
comprising at least one lysine residue and/or at least one arginine residue; and

wherein AC is an antisense compound; and

(d) a therapeutic moiety (TM).

[0004b] In another aspect of the present disclosure, there is provided a compound comprising:

(a) a cyclic cell penetrating peptide (CPP) of Formula (I):



or a protonated form thereof,

wherein:

three of R₁, R₂, and R₃ are -CH₂Ph;

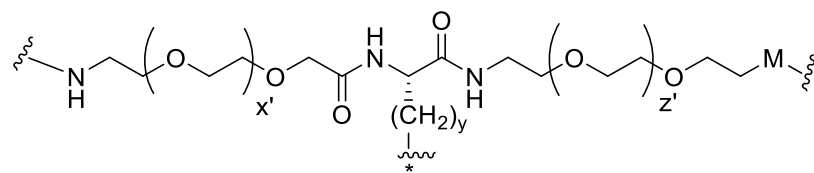
R₄ and R₆ are independently H or an amino acid side chain;

AA_{SC} is an amino acid side chain;

q is 1, 2, 3 or 4; and

each m is independently an integer 0, 1, 2, or 3;

(b) a linker comprising the structure



wherein:

x' is an integer from 1-23;

y is an integer from 1-5;

z' is an integer from 1-23;

* is the point of attachment to the AA_{SC}; and

M is a bonding group;

(c) an exocyclic peptide (EP) comprising from 2 to 10 amino acid residues and comprising at least one lysine residue and/or at least one arginine residue; and wherein AC is an antisense compound; and

(d) a therapeutic moiety (TM).

[0004c] In yet another aspect of the present disclosure, there is provided a pharmaceutical composition comprising the compound as disclosed herein.

10 **[0004d]** In a further aspect of the present disclosure, there is provided a use of a compound as disclosed herein or a pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating a disease or disorder in a subject.

[0004e] In another aspect of the present disclosure, there is provided a compound as disclosed herein, or a pharmaceutical composition as disclosed herein, for use in therapy.

15 **[0005]** Compositions and methods for modulating tissue distribution and/or retention of intracellular therapeutic agents are described herein. Compounds comprising a cell penetrating peptide (CPP) linked to a therapeutic moiety (TM) have been found to have altered tissue distribution and/or retention when the compound further comprises an exocyclic peptide (EP) as described herein. The EP typically is a lysine-containing peptide. EPs have been identified that
20 previously were known in the art as “nuclear localization signals” (NLS), such as the nuclear localization sequence of the SV40 virus large T-antigen, the minimal functional unit of which is the seven amino acid sequence PKKKRKV. Inclusion of an EP in the CPP-TM compound can, for example, alter the level of expression, activity or function of the TM in different tissues, such as different types of muscle tissue or different types of central nervous system tissue (see e.g.,
25 Examples 5 and 7). In one example, the compound is administered intrathecally and the tissue distribution and/or retention of the compound in tissues of the CNS is modulated.

[0006] In examples, a compound is provided that comprises:

(a) a cell penetrating peptide (CPP);

(b) a therapeutic moiety (TM); and

(c) an exocyclic peptide (EP), wherein tissue distribution or retention of the compound is

5 modulated as compared to a compound comprising the CPP and TM but lacking the EP.

[0007] In one example, the EP is conjugated to the CPP. In one example, the EP is conjugated to the TM. In one example, the CPP is a cyclic cell penetrating peptide (cCPP). In various examples, the therapeutic moiety is a protein, a polypeptide, an oligonucleotide or a

small molecule. In one example, the oligonucleotide is an antisense compound (AC). In one example, the oligonucleotide is other than an antisense compound (AC).

[0008] In examples, the therapeutic compound comprises an AC that modulates splicing of exon 2, 8, 11, 17, 19, 23, 29, 40, 41, 42, 43, 44, 45, 46, 48, 49, 50, 51, 52, 53, 55, and 59 of DMD. In examples, the compound comprises an AC that modulates splicing of exon 2, 8, 11, 23 43, 44, 45, 50, 51, 53, and 55 of DMD. In examples, the compound comprises an AC that modulates splicing of exon 2, 23, 44, or 51 of DMD.

[0009] In examples, the compound comprises an AC that modulates splicing of exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7a, and exon 7b of CD33.

[0010] In examples, the compound is selected from the group consisting of EEV-PMO-MDX23-1,2,3, EEV-PMO-CD33-1 and the compounds shown in Table C of Example 5, having the structures shown herein.

[0011] In examples, a pharmaceutical composition is provided that includes the compound described herein and a pharmaceutically acceptable carrier.

[0012] In examples, a cell is provided that includes a compound described herein.

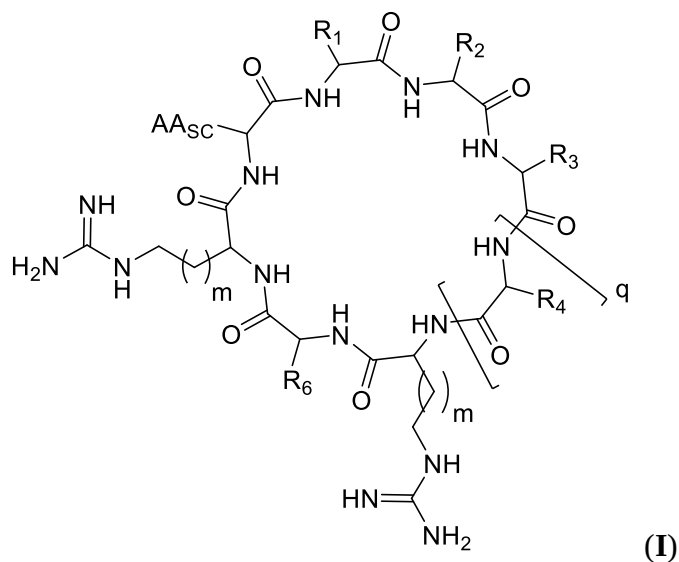
[0013] The present disclosure also relates to a method of modulating tissue distribution or retention of a therapeutic agent in a subject in need thereof, comprising administering a compound of the present disclosure. In examples, the compound is administered to the subject intrathecally and the compound modulates tissue distribution or retention of the therapeutic agent in tissues of the central nervous system (CNS). In examples, the compound modulates tissue distribution or retention of the therapeutic agent in muscle tissue.

[0014] The present disclosure also pertains to a method of treating a disease or disorder in a subject in need thereof, comprising administering a compound of the present disclosure. In examples, the therapeutic agent is an antisense compound (AC) and administration of the compound modulates splicing or expression of a target gene, degrades mRNA, stabilizes mRNA, or sterically blocks mRNA. In examples, administration of the compound modulates splicing of the target pre-mRNA.

CLAIMS

1. A compound comprising:

(a) a cyclic cell penetrating peptide (CPP) of Formula (I):



or a protonated form thereof,

wherein:

one of R_1 , R_2 , and R_3 are H;

two of R_1 , R_2 , and R_3 are $-\text{CH}_2\text{Ph}$;

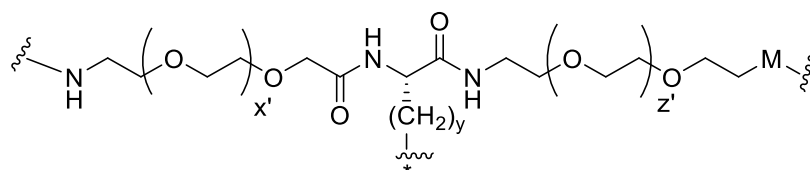
R_4 and R_6 are independently H or an amino acid side chain;

AA_{SC} is an amino acid side chain;

q is 1, 2, 3 or 4; and

each m is independently an integer 0, 1, 2, or 3;

(b) a linker comprising the structure



wherein:

x' is an integer from 1-23;

y is an integer from 1-5;

z' is an integer from 1-23;

$*$ is the point of attachment to the AA_{SC}, and

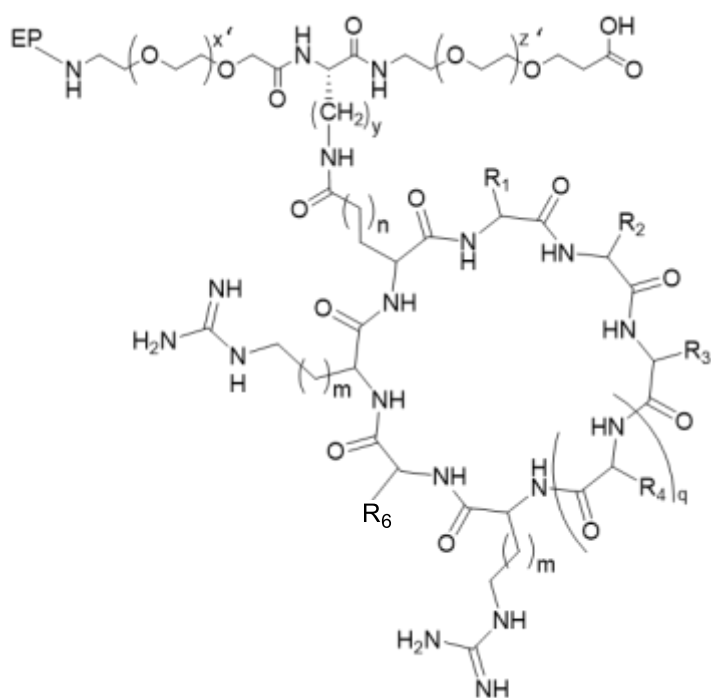
M is a bonding group;

(c) an exocyclic peptide (EP) comprising from 2 to 10 amino acid residues and comprising at least one lysine residue and/or at least one arginine residue; and

wherein AC is an antisense compound; and

(d) a therapeutic moiety (TM).

2. The compound of claim 1, comprising Formula (B):



(B), or a protonated form thereof.

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wherein:

R₄ and R₆ are independently H or an amino acid side chain;

q is 1, 2, 3 or 4; and

each m is independently an integer 0, 1, 2, or 3;

wherein:



y is an integer from 1-5;

z' is an integer from 1-23;

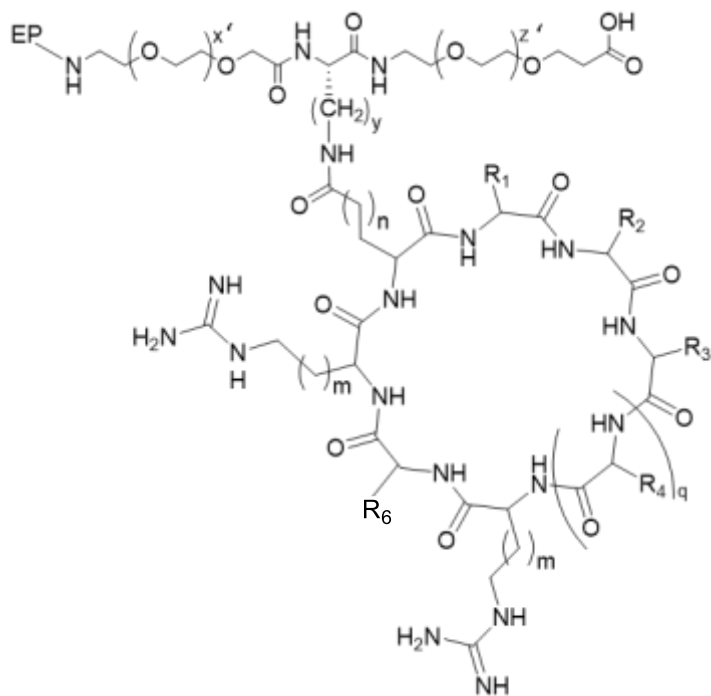
* is the point of attachment to the AA_{SC} ; and

M is a bonding group;

232

comprising at least one lysine residue and/or at least one arginine residue; and
 wherein AC is an antisense compound; and
 (d) a therapeutic moiety (TM).

- 5 4. The compound of claim 3, comprising Formula (B):



(B), or a protonated form thereof.

5. The compound of any of claims 1-4, wherein the therapeutic moiety is a protein, a polypeptide, a small molecule or an oligonucleotide other than an antisense compound.

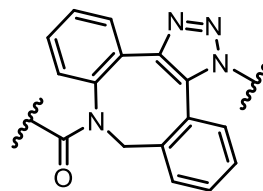
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6. The compound of any of claims 1-5, wherein the therapeutic moiety is an antisense compound (AC).

7. The compound of any of claims 1, 3 or 5-6 wherein M is -C(O).

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8. The compound of any of claims 1, 3 or 5-6, wherein M is



9. The compound of any of claims 1-8, wherein z is 11.

10. The compound of any one of claims 1-9, wherein x is 1.

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11. The compound of any of claims 1-10, wherein the EP comprises from 4 to 8 amino acid residues.

12. The compound of any of claims 1-11, wherein the EP comprises 1, 2, 3, or 4 lysine residues.

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13. The compound of any of claims 1-12, wherein the EP comprises at least 2 amino acid residues with a hydrophobic side chain selected from valine, proline, alanine, leucine, isoleucine, or methionine.

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14. The compound of any one of claims 1-11, wherein the exocyclic peptide comprises one of the following sequences: PKKKRKV; KR; RR; KKK; KGK; KBK; KBR; KRK; KRR; RKK; RRR; KKKK; KKRK; KRKK; KRRK; RKKR; RRRR; KGKK; KKGK; KKKKK; KKKRK; KBKKBK; KKKRKV; PGKKRKV; PKGKRKV; PKKGRKV; PKKKGKV; PKKKRGV; or PKKKRKG.

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15. The compound of any one of claims 1-14, wherein the exocyclic peptide has the structure: Ac-PKKKRKV-.

16. The compounds of any of claims 1-2 and 5-15, wherein R₁ is the side chain of phenylalanine.

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17. The compound of any of claims 1-2 and 5-15, wherein R₂ is the side chain of phenylalanine.

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18. The compound of any of claims 1-2 and 5-15, wherein R₃ is the side chain of phenylalanine.
19. The compound of any of claims 1-18, wherein R₄ is H.
20. The compound of any of claims 1-19, wherein R₆ is H.
21. The compound of any of claims 1-20, wherein the cCPP comprises one to ten D amino acids.
22. The compound of any of claims 1-20, wherein the cCPP comprises all L-amino acids.
23. The compound of any of claims 1-20, wherein the cCPP comprises all D-amino acids.
24. The compound of any of claims 1-23, wherein q is 1.
25. The compound of any of claims 1-24, wherein m is 1
26. The compound of any one of claims 1-2 or 4-25, wherein the cCPP is selected from:
FGFGRGRQ, GfFGrGrQ, FGFGRRRQ and FGFRRRRQ.
27. The compound of claim 1 or 2, wherein the compound has a structure selected from:
Ac-PKKKRKV-miniPEG₂-K (*cyclo*(GfFGrGrQ))-PEG₁₂-OH;
Ac-PKKKRKV-miniPEG₂-K(*cyclo*[FGFKRKRQ])-PEG₁₂-OH;
Ac-PKKKRKV-miniPEG₂-K(*cyclo*[FGFRGRGQ])-PEG₁₂-OH;
Ac-PKKKRKV-miniPEG₂-K(*cyclo*[FGFGRGRGRQ])-PEG₁₂-OH;
Ac-PKKKRKV-miniPEG₂-K(*cyclo*[FGFGRrRQ])-PEG₁₂-OH;
Ac-PKKKRKV-miniPEG₂-K(*cyclo*[FGFGRRRQ])-PEG₁₂-OH; and
Ac-PKKKRKV-miniPEG₂-K(*cyclo*[FGFRRRRQ])-PEG₁₂-OH.

28. The compound of claim 1 or 2, wherein the compound has a structure selected from:

Ac-KKKRKG-miniPEG₂-K(*cyclo*[FGFGRGRQ])-PEG₁₂-OH;

Ac-KKKRK-miniPEG₂-K(*cyclo*[FGFGRGRQ])-PEG₁₂-OH;

Ac-KKRKK-PEG₄-K(*cyclo*[FGFGRGRQ])-PEG₁₂-OH;

5 Ac-KRKKK-PEG₄-K(*cyclo*[FGFGRGRQ])-PEG₁₂-OH;

Ac-KKKKR-PEG₄-K(*cyclo*[FGFGRGRQ])-PEG₁₂-OH;

Ac-RKKKK-PEG₄-K(*cyclo*[FGFGRGRQ])-PEG₁₂-OH; and

Ac-KKKRK-PEG₄-K(*cyclo*[FGFGRGRQ])-PEG₁₂-OH.

10 29. The compound of claim 1 or 2, wherein the compound has a structure selected from:

Ac-PKKKRKV-PEG₂-K(*cyclo*[FGFGRGRQ])-PEG₂-K(N₃)-NH₂;

Ac-PKKKRKV-PEG₂-K(*cyclo*[FGFGRGRQ])-PEG₁₂-OH;

Ac-PKKKRKV-PEG₂-K(*cyclo*[GfFGrGrQ])-PEG₂-K(N₃)-NH₂; and

Ac- PKKKRKV-PEG₂-K(*cyclo*[GfFGrGrQ])-PEG₁₂-OH.

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30. The compound of claim 1 or 2, wherein the compound has a structure selected from:

Ac-PKKKRKV-PEG₂-K(*cyclo*[FGFGRGRQ])-PEG₁₂-OH;

Ac-PKKKRKV-PEG₂-K(*cyclo*[GfFGrGrQ])-PEG₁₂-OH;

Ac-PKKKRKV-PEG₂-K(*cyclo*[FGFRRRRQ])-PEG₁₂-OH; and

20 Ac-PKKKRKV-PEG₂-K(*cyclo*[FGFRRRRQ])-PEG₁₂-OH.

31. The compound of claim 1 or 2, wherein the compound has the structure:

Ac-PKKKRKV-AEEA-K(*cyclo*[FGFGRGRQ])-PEG₁₂-OH; or

Ac-PKKKRKV-AEEA-K(*cyclo*[GfFGrGrQ])-PEG₁₂-OH.

25

32. The compound of any one of claims 3-23, wherein the cCPP is selected from:

FfFGRGRQ and FfFGrGrQ.

33. The compound of claim 3 or 4, wherein the compound has a structure selected from:
 Ac-PKKKRKV-miniPEG₂-K(cyclo(FfFGRGRQ))-miniPEG₂-K(N₃)-NH₂;
 Ac-PKKKRKV-PEG₂-K(cyclo[FfFGRGRQ])-PEG₁₂-OH;
 Ac-KKKK-miniPEG₂-K(cyclo(FfFGrGrQ))-miniPEG₂-K(N₃)-NH₂;
 5 Ac-KKKK-miniPEG₂-K(cyclo(FfFGrGrQ))-miniPEG₂-K(N₃)-NH₂;
 Ac-KBKBK-miniPEG₂-K(cyclo(FfFGrGrQ))-miniPEG₂-K(N₃)-NH₂;
 Ac-PKKKRKV-miniPEG₂-K(cyclo(FfFGrGrQ))-miniPEG₂-K(N₃)-NH₂;
 Ac-KGKK-miniPEG₂-K(cyclo(FfFGrGrQ))-miniPEG₂-K(N₃)-NH₂; and
 Ac-KGKK-miniPEG₂-K(cyclo(FfFGrGrQ))-miniPEG₂-K(N₃)-NH₂.
- 10
34. The compound of any one of the preceding claims, wherein the compound modulates splicing of exon 2, 8, 11, 23 43, 44, 45, 50, 51, 53, and 55 of DMD.
35. The compound of any one of the preceding claims, wherein the compound modulates
 15 splicing of exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7a, and exon 7b of CD33.
36. A pharmaceutical composition comprising the compound of any one of claims 1-35.
37. Use of a compound of any of claims 1-35 or a pharmaceutical composition of claim 36,
 20 in the manufacture of a medicament for treating a disease or disorder in a subject.
38. The use of claim 37, wherein the medicament is to be subcutaneously, intradermally, intravenously, intramuscularly, intraperitoneally, or intrasternally administered to the subject.
- 25 39. The use of claim 37, wherein the medicament is to be intrathecally administered to the subject.
40. The use of any one of claims 37-39, wherein the disease or disorder is a central nervous system disorder, a neuromuscular disorder, or a musculoskeletal disorder.
- 30 41. The use of claim 37, wherein the disease is Duchenne muscular dystrophy.

42. The use of claim 37, wherein the disease or disorder comprises a neuroinflammatory disease selected from Alzheimer's disease or Parkinson's disease.

5 43. A compound of any of claims 1-35, or a pharmaceutical composition of claim 36, for use in therapy.