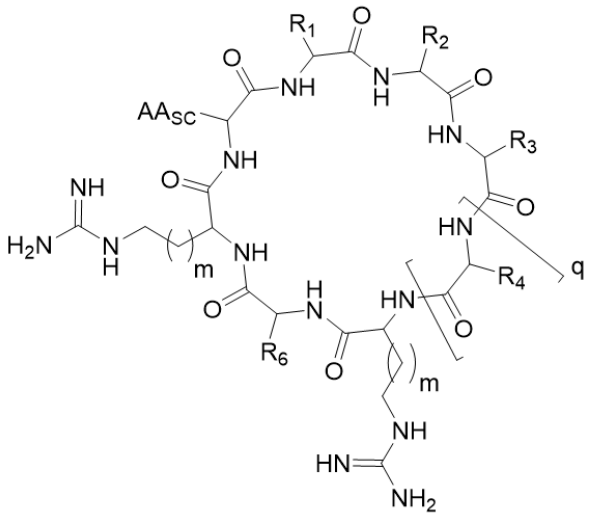
low and there remains a need for improved delivery systems to increase the potency of these antisense compounds.

[0005] There is an unmet need for effective compositions to deliver antisense compounds to intracellular compartments to treat DMD.

SUMMARY

[0005a] In an aspect of the present disclosure, there is provided a compound comprising an endosomal escape vehicle (EEV) conjugated to a therapeutic moiety comprising an antisense compound (AC) that is complementary to a target sequence comprising at least a portion of exon 45 of *DMD* gene in a pre-mRNA sequence; wherein the EEV comprises:

(a) a cyclic peptide of formula:



, or a protonated form thereof,

wherein

R_1, R_2, and R_3 are each independently H or a side chain comprising an aryl or heteroaryl group;

at least two of R_1, R_2, and R_3 are a side chain of phenylalanine;

AA_{SC} is an amino acid side chain;

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

each m is independently 0, 1, 2, or 3; and

q is 1, 2, 3 or 4;

(b) an exocyclic peptide (EP) comprising from 2 to 10 amino acid residues, wherein 1 or 2 amino acid residues comprise a side chain of a guanidine group, or a protonated form thereof or 2, 3, or 4 lysine residues;

(c) a linker comprising:

(i) a -$(OCH_2CH_2)_z$- subunit, wherein z' is an integer from 1 to 23;

(ii) one or more amino acid residues, such as a residue of glycine, β-alanine, 4-aminobutyric acid, 5-aminopentoic acid or 6-aminohexanoic acid, or combinations thereof; or

(iii) combinations of (i) and (ii).

[0006] Compositions for delivering nucleic acids are described herein. In examples, the nucleic acids are antisense compounds (AC). In examples, the antisense compounds target exon 45 in a subject with Duchenne muscular dystrophy (DMD).

[0007] In examples, a compound is provided that comprises:

(a) a cyclic peptide (also referred to herein as a cell pepentrating peptide or "CPP"); and

(b) an antisense compound (AC) that is complementary to a target sequence of a *DMD* gene in a pre-mRNA sequence, wherein the target sequence comprises at least a portion of the 5' flanking intron of exon 45, at least a portion of exon 45, at least a portion of the 3' flanking intron of exon 45, or a combination thereof.

[0008] In examples, the AC comprises at least one modified nucleotide or nucleic acid selected from a phosphorothioate (PS) nucleotide, a phosphorodiamidate morpholino (PMO) nucleotide, a locked nucleic acid (LNA), a peptide nucleic acid (PNA), a nucleotide comprising a 2'-O-methyl (2'-OMe) modified backbone, a 2'O-methoxy-ethyl (2'-MOE) nucleotide, a 2',4' constrained ethyl (cEt) nucleotide, and a 2'-deoxy-2'-fluoro-beta-D-arabinonucleic acid (2'F-ANA). In examples, the AC comprises at least one PMO (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 PMO, inclusive of all ranges therein). In examples, each nucleotide in the AC is a PMO.

[0009] In examples, the AC comprises the sequence: 5'-ATGCCATCCTGGAGTTCCTGTA-3'. In examples, the AC comprises the sequence: 5'-CCCAATGCCATCCTGGAGTTCCT-3'.

[0010] In examples, the cyclic peptide is FGFGRGRQ. In examples, the cyclic peptide is GfFGrGrQ. In examples, the cyclic peptide is FfΦGRGRQ.

[0011] In examples, the EEV is: Ac-PKKKRKV-AEEA-Lys-(cyclo[FGFGRGRQ])-PEG12-OH.

[0012] In examples, provided herein is a pharmaceutical composition comprising a compound described herein.

[0013] In examples, provided herein is a cell comprising a compound described herein.

[0014] In examples, provided herein is a method of treating DMD comprising administering a compound described herein to a patient in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] **FIGs. 1A** and **1B** shows a conjugation chemistry for connecting an antisense compound (AC) with a cell penetrating peptide (CPP).

[0016] **FIGs. 2A-2B** shows a conjugation chemistry for connecting a cell penetrating peptide (CPP), shown as 

, and an antisense compound (AC), wherein the CPP includes a PEG4 linker and the AC is shown without (FIG. 2A) and with (FIG. 2B) a linker containing a polyethylene glycol (PEG2 or miniPEG) moiety. "R" in the figure represents a palmitoyl group.

[0017] **FIG. 3** shows examples of endosomal escape vehicle (EEV) design using a representative CPP. It is understood that the CPP can include any of the CPP disclosed herein.

[0018] **FIG. 4** shows the exon 45 skipping efficiencies of select ACs from **Table 8** at 5 μM and 10 μM.

[0019] **FIGs. 5A-5D** show exon skipping assessment in the triceps (**FIG. 5A**), tibialis anterior (**FIG. 5B**), diaphragm (**FIG. 5C**), and heart (**FIG. 5D**) 1 week post administration (intravenous) with EEV-PMO-MDX23-1 as assessed by 2-Step RT-PCR.

[0020] **FIG.s 6A-6D** show the duration of effect (1 week, 2 weeks, 4 weeks and 8 weeks) in the triceps (**FIG. 6A**), tibialis anterior (**FIG. 6B**), diaphragm (**FIG. 6C**), and heart (**FIG. 6D**) after intravenous administration of 80 mpk EEV-PMO-MDX23-1.

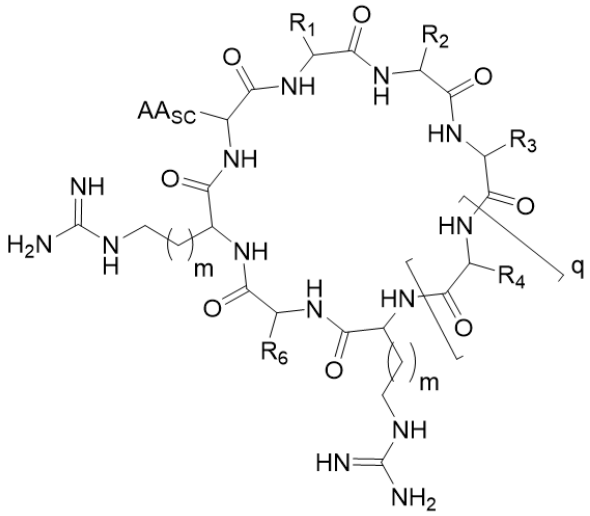
[0021] **FIG. 7** depicts results after intravenous dosing of 40 mpk EEV-PMO-MDX23-1 (4 weekly doses).

[0022] **FIG. 8** shows D2.mdx wire hang data. After 12 weeks of treatment, animals treated with EEV-PMO-MDX23-1 80mpk Q2W had a wire hang time that was statistically indistinguishable from the WT animals. DBA WT Vehicle (saline); D2.mdx Vehicle (saline); D2.mdx EEV-PMO-MDX23-1; D2.mdx EEV-PMO-MDX23-2; D2.mdx PMO-MDX23 (5'-GGCCAAACCTCGGCTTACCTGAAAT-3')

CLAIMS

1. A compound comprising an endosomal escape vehicle (EEV) conjugated to a therapeutic moiety comprising an antisense compound (AC) that is complementary to a target sequence comprising at least a portion of exon 45 of *DMD* gene in a pre-mRNA sequence; wherein the EEV comprises:

(a) a cyclic peptide of formula:



, or a protonated form thereof,

wherein

R_1, R_2, and R_3 are each independently H or a side chain comprising an aryl or heteroaryl group;

at least two of R_1, R_2, and R_3 are a side chain of phenylalanine;

AA_{SC} is an amino acid side chain;

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

each m is independently 0, 1, 2, or 3; and

q is 1, 2, 3 or 4;

(b) an exocyclic peptide (EP) comprising from 2 to 10 amino acid residues, wherein 1 or 2 amino acid residues comprise a side chain of a guanidine group, or a protonated form thereof or 2, 3, or 4 lysine residues;

(c) a linker comprising:

(i) a $-(OCH_2CH_2)_z-$ subunit, wherein z' is an integer from 1 to 23;

(ii) one or more amino acid residues, such as a residue of glycine, β-

alanine, 4-aminobutyric acid, 5-aminopentoic acid or 6-aminohexanoic acid, or combinations thereof; or

(iii) combinations of (i) and (ii).

2. The compound of claim 1, wherein the AC comprises at least one modified nucleotide or nucleic acid comprising phosphorothioate (PS) nucleotide, a phosphorodiamidate morpholino nucleotide (PMO), a locked nucleic acid (LNA), a peptide nucleic acid (PNA), a nucleotide comprising a 2’-O-methyl (2’-OMe) modified backbone, a 2’O-methoxy-ethyl (2’-MOE) nucleotide, a 2',4' constrained ethyl (cEt) nucleotide, ora 2'-deoxy-2'-fluoro-beta-D-arabinonucleic acid (2'F-ANA).

3. The compound of claim 1, wherein the AC comprises 15-30 nucleotides.

4. The compound of claim 1, wherein the cyclic peptide is conjugated to the 3' end of the AC, the 5’ end of the AC, or the backbone of the AC.

5. The compound of claim 1, wherein the linker conjugates the cyclic peptide to the AC.

6. The compound of claim 1, wherein the AC comprises a sequence with at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence comprising 5’- CCCAATGCCATCCTGGAGTTCCT -3’.

7. The compound of claim 6, wherein the AC comprises a sequence comprising at least 15, 16, 17, 18, 19, 20, 21, 22, or 23 consecutive nucleotides of a base sequence comprising: 5’-CCCAATGCCATCCTGGAGTTCCT -3’.

8. The compound of claim 1, wherein the AC has a sequence comprising:

5’- AATGCCATCCTGGAGTTCCTG -3’;

5’- ATGCCATCCTGGAGTTCCTGT -3’;

5’- CCCAATGCCATCCTGGAGTTCC -3’;

5’- ATGCCATCCTGGAGTTCCTGTA -3’;

5’- GCCCAATGCCATCCTGGAGTTCC -3’;

5’- CCCAATGCCATCCTGGAGTTCCT -3’;

5’- CCCAATGCCATCCTGGAGTTCCTG -3’;

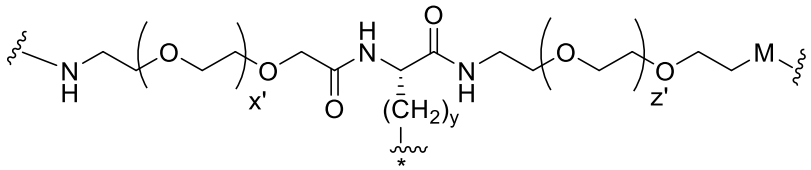
5’- TGCCCAATGCCATCCTGGAGTTCCT -3’;

5’- CCCAATGCCATCCTGGAGTTCCTGT -3’;

5’- CAATGCCATCCTGGAGTTCCTGT -3’; or

any reverse complement thereof.

9. The compound of claim 1, wherein the linker is of formula:



, wherein

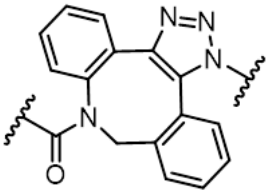
x’ is an integer from 1-23;

y is an integer from 1-5;

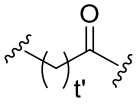
z’ is an integer from 1-23;

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

M is a bonding group.

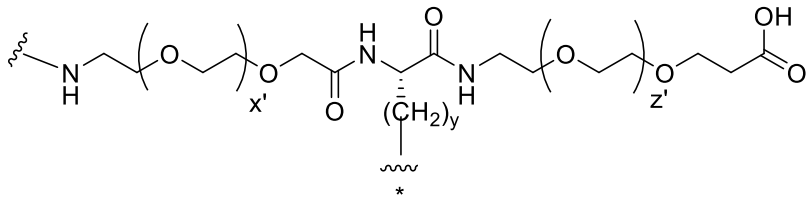
10. The compound of claim 9, wherein M comprises 

, or



, wherein t’ is an integer from 0 to 10.

11. The compound of claim 9, wherein the linker has the formula:



, wherein:

z’ is 11;

x’ is 1; or

z’ is 11 and x’ is 1.

12. The compound of claim 1, wherein R_4 and R_6 are independently an amino acid side chain of citrulline or serine.

13. The compound of claim 1 wherein:

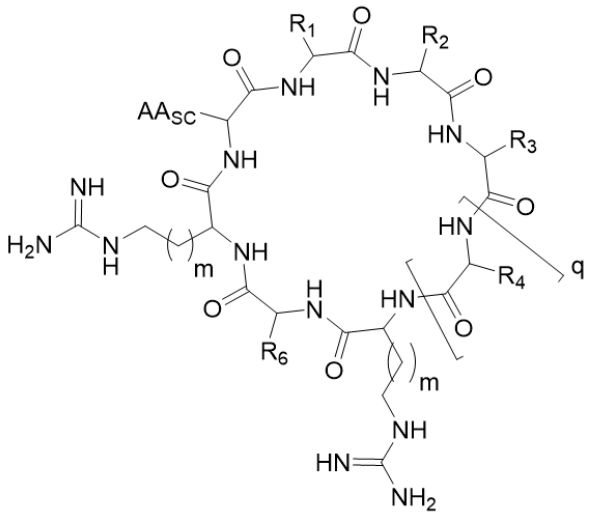
(i) the exocyclic peptide comprises 1 or 2 amino acid residues comprising a side chain comprising a guanidine group, or a protonated form thereof;

(ii) the exocyclic peptide comprises 2, 3, or 4 lysine residues; and

(iii) the exocyclic peptide comprises at least 2 amino acid residues with a hydrophobic side chain, for example wherein the hydrophobic side chain is selected from valine, proline, alanine, leucine, isoleucine, and methionine.

14. The compound of claim 1, wherein the EP has the structure: Ac-PKKKRKV.

15. The compound of claim 1, wherein the cyclic peptide comprises:



, or a protonated form thereof,

wherein,

one of R_1, R_2, and R_3 is H;

two of R_1, R_2, and R_3 are CH_2Ph;

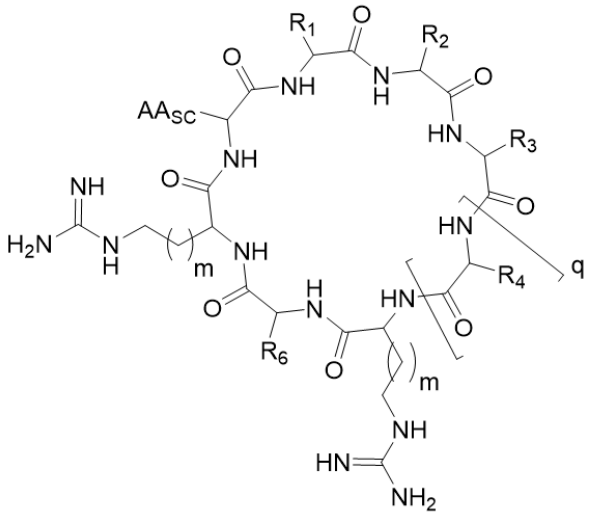
R_4 and R_6 are independently H or an amino acid side chain;

AA_{sc} is an amino acid side chain;

q is 1, 2, 3 or 4; and

each m is independently 0, 1, 2, or 3.

16. The compound of claim 1, wherein the cyclic peptide comprises:



, or a protonated form thereof,

wherein,

R_1, R_2, and R_3 are -CH_2Ph;

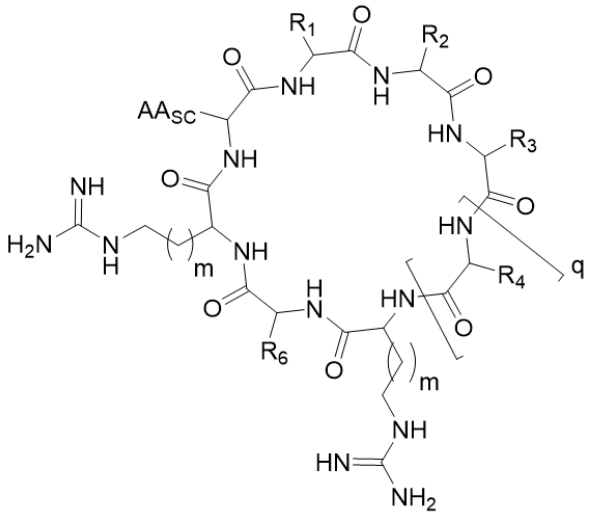
R_4 and R_6 are independently H or an amino acid side chain;

AA_{sc} is an amino acid side chain;

q is 1, 2, 3 or 4; and

each m is independently 0, 1, 2, or 3.

17. The compound of claim 1, wherein the cyclic peptide comprises:



, or a protonated form thereof,

wherein,

R_1, R_2, and R_3 are each independently a side chain comprising an aryl or heteroaryl group;

at least two of R_1, R_2, and R_3 are a side chain of phenylalanine;

AA_{SC} is an amino acid side chain;

R_4 and R_6 are independently H or an amino acid side chain of serine or citrulline

q is 1, 2, 3 or 4; and

each m is independently 0, 1, 2, or 3.

18. The compound of claim 1, wherein the cyclic peptide is selected from Ff-Nal-GrGrQ; Ff-Nal-GRGRQ; FfFGRGRQ; FGFGRGRQ; GfFGrGrQ; FGFGRRRQ; and FGFRRRRQ.

19. The compound of claim 1, wherein the EEV has the formula:

Ac-PKKKRKV-PEG_x-K(*cyclo*[FGFGRGRQ])-PEG_y-K(N_3)-NH_2;

Ac-PKKKRKV-PEG_x-K(*cyclo*[FGFGRGRQ])-PEG_y-OH;

Ac-PKKKRKV-PEG_x-K(*cyclo*[GfFGrGrQ])-PEG_y-K(N_3)-NH_2;

Ac-PKKKRKV-PEG_x-K(*cyclo*[GfFGrGrQ])-PEG_y-OH;

Ac-PKKKRKV-PEG_x-K(*cyclo*[FfFGRGRQ])-PEG_y-K(N_3)-NH_2;

Ac-PKKKRKV-PEG_x-K(*cyclo*[FfFGRGRQ])-PEG_y-OH;

Ac-PKKKRKV-PEG_x-K(*cyclo*[Ff-Nal-GrGrQ])-PEG_y-K(N_3)-NH_2; or

Ac-PKKKRKV-PEG_x-K(*cyclo*[Ff-Nal-GrGrQ])-PEG_y-OH;

prior to conjugation to the therapeutic moiety.

20. The compound of claim 1, wherein the EEV has the formula:

Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFGRGRQ])-PEG_2-K(N_3)-NH_2;

Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFGRGRQ])-PEG_{12}-K(N_3)-NH_2;

Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFGRGRQ])-PEG_2-OH;

Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFGRGRQ])-PEG_{12}-OH;

Ac-PKKKRKV-PEG_2-K(*cyclo*[GfFGrGrQ])-PEG_2-K(N_3)-NH_2;

Ac-PKKKRKV-PEG_2-K(*cyclo*[GfFGrGrQ])-PEG_{12}-K(N_3)-NH_2;

Ac-PKKKRKV-PEG_2-K(*cyclo*[GfFGrGrQ])-PEG_2-OH;

Ac-PKKKRKV-PEG_2-K(*cyclo*[GfFGrGrQ])-PEG_{12}-OH;

Ac-PKKKRKV-PEG_2-K(*cyclo*[FfFGRGRQ])-PEG_2-K(N_3)-NH_2;

Ac-PKKKRKV-PEG_2-K(*cyclo*[FfFGRGRQ])-PEG_{12}-K(N_3)-NH_2;

Ac-PKKKRKV-PEG_2-K(*cyclo*(FfFGRGRQ)-PEG_2-OH;

Ac-PKKKRKV-PEG_2-K(*cyclo*(FfFGRGRQ)-PEG_{12}-OH;

Ac-PKKKRKV-PEG_2-K(*cyclo*[Ff-Nal-GrGrQ])-PEG_2-K(N_3)-NH_2;

Ac-PKKKRKV-PEG_2-K(*cyclo*[Ff-Nal-GrGrQ])-PEG_{12}-K(N_3)-NH_2;

Ac-PKKKRKV-PEG_2-K(*cyclo*[Ff-Nal-GrGrQ])-PEG_2-OH;

Ac-PKKKRKV-PEG_2-K(*cyclo*[Ff-Nal-GrGrQ])-PEG_{12}-OH;

Ac-PKKKRKV-PEG_2-K(*cyclo*[Ff-Φ-Cit-r-Cit-r-Q])-PEG_{12}-K(N_3)-NH_2;

Ac-PKKKRKV-K(*cyclo*[FfΦ-R-r-Cit-rQ])-PEG_{12}-K(N_3)-NH_2;

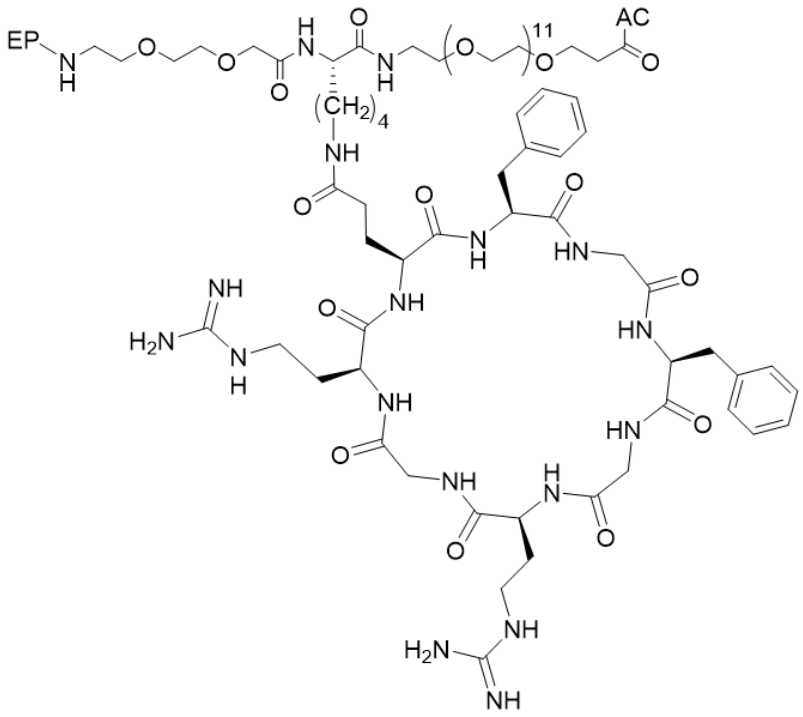
Ac-PKKKRKV-K(*cyclo*[FfΦ-Cit-r-R-rQ])-PEG_{12}-K(N_3)-NH_2;

Ac-PKKKRKV-K(*cyclo*[FfΦR-cit-R-cit-Q])-PEG_{12}-K(N_3)-NH_2;

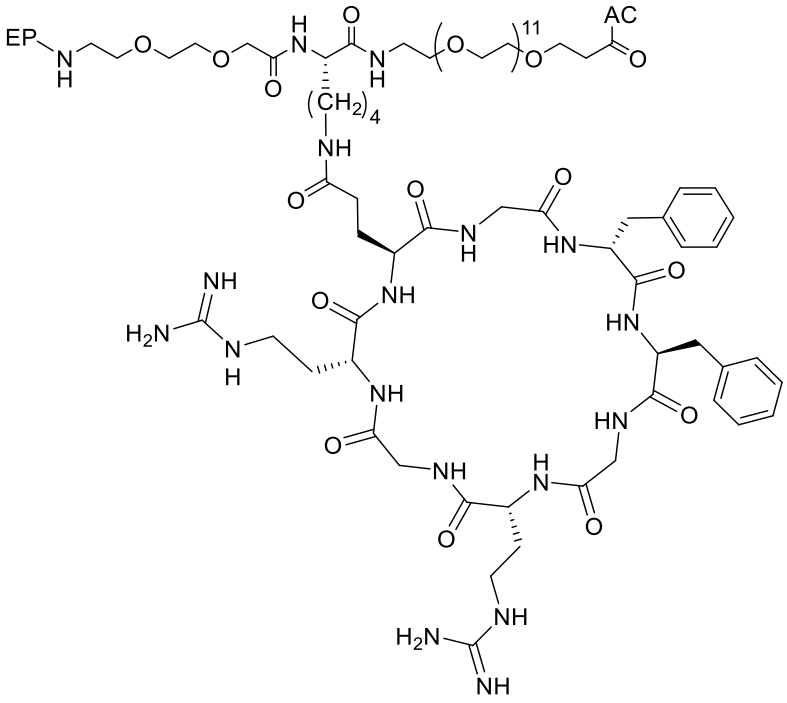
Ac-PKKKRKV-PEG_2-K(*cyclo*[FfΦ-Cit-r-Cit-rQ])-PEG_2-k(N_3)-NH_2;
Ac-PKKKRKV-PEG_2-K(*cyclo*[FfΦ-Cit-r-Cit-rQ])-PEG_4-k(N_3)-NH_2;
Ac-PKKKRKV-K(cyclo[FfΦ-Cit-r-Cit-rQ])-PEG_{12}-k(N_3)-NH_2;
Ac-PKKKRKV-PEG_2-K(*cyclo*[FfΦ-Cit-r-Cit-r-Q])-PEG_{12}-k(N_3)-NH_2; or
Ac-PKKKRKV-PEG_2-K(*cyclo*[FfΦ-Cit-r-Cit-r-Q])-PEG_{12}-K(N_3)-NH_2;
prior to conjugation to the therapeutic moiety.

21. The compound of claim 1, wherein the EEV has the formula:
Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFGRGRQ])-PEG_{12}-OH;
Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFGRGRQ])-PEG_2-K(N_3)-NH_2;
Ac-PKKKRKV-PEG_2-K(*cyclo*[GfFGrGrQ])-PEG_{12}-OH;
Ac-PKKKRKV-PEG_2-K(*cyclo*[GfFGrGrQ])-PEG_2-K(N_3)-NH_2;
Ac-PKKKRKV-K(cyclo[Ff-Nal-RrRrQ])-PEG_{12}-K(N_3)-NH_2;
Ac-PKKKRKV-PEG_2-K(*cyclo*[FfFGRGRQ])-PEG_2-K(N3)-NH_2;
Ac-PKKKRKV-K(*cyclo*[Ff-Nal-GrGrQ])-PEG_{12}-K(N_3)-NH_2; or
Ac-PKKKRKV-PEG_2-K(*cyclo*[Ff-Nal-GrGrQ])-PEG_{12}-OH;
prior to conjugation to the therapeutic moiety.

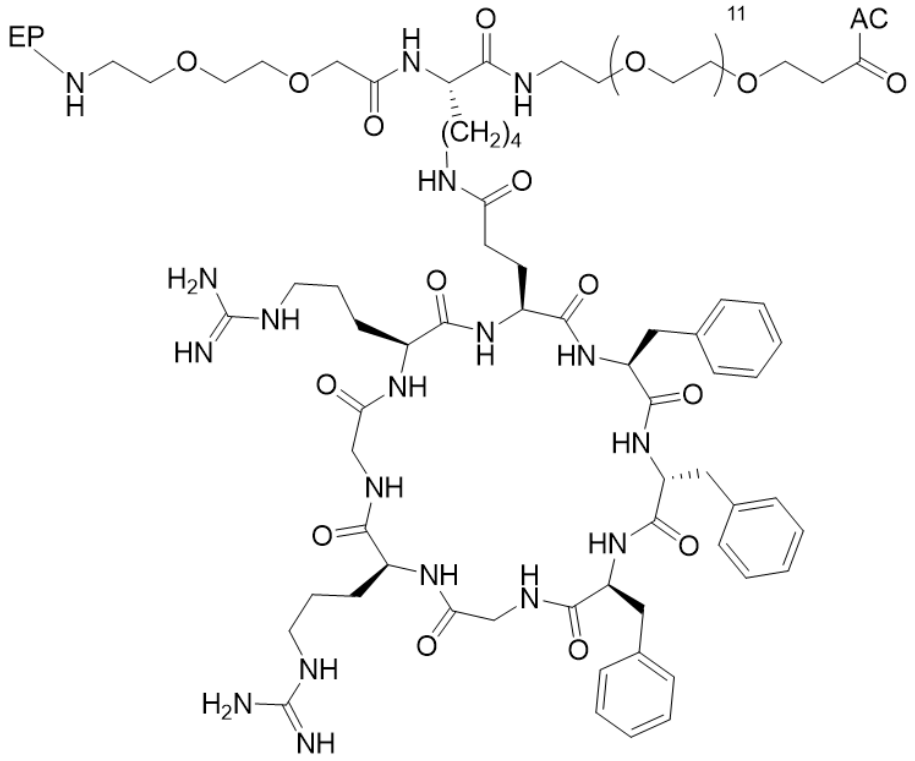
22. The compound of any one of claims 1 to 8, 13, or 14, wherein the Endosomal Escape Vehicle (EEV) conjugated to a therapeutic moiety comprising an antisense compound (AC) comprises the structure of Formula (**C-1**), Formula (**C-2**), Formula (**C-3**), or Formula (**C-4**):



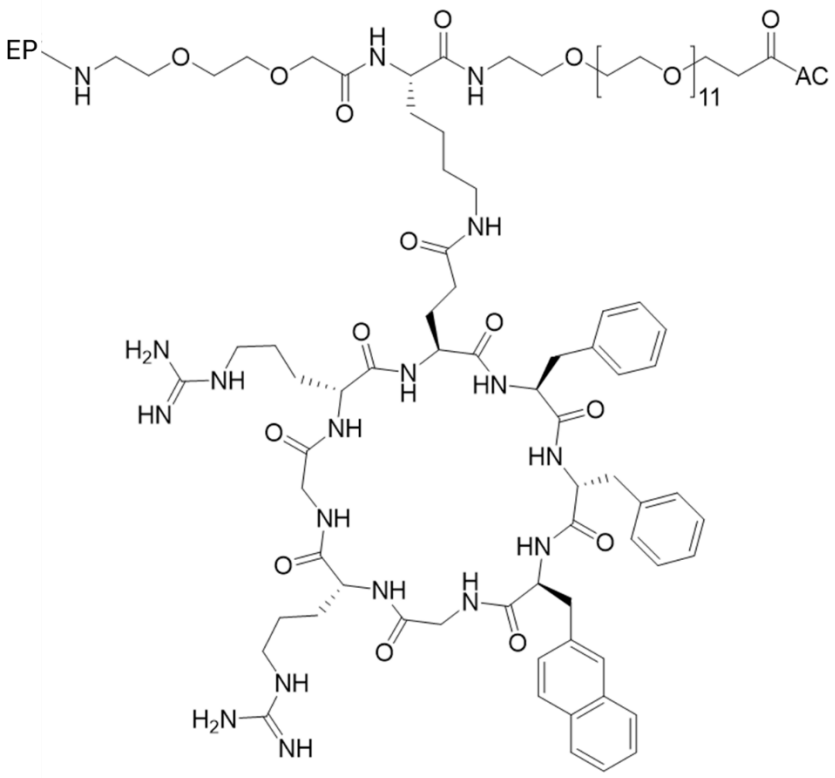
(**C-1**),



(**C-2**),



(**C-3**),



(**C-4**)

or a protonated form thereof, wherein EP is the exocyclic peptide.