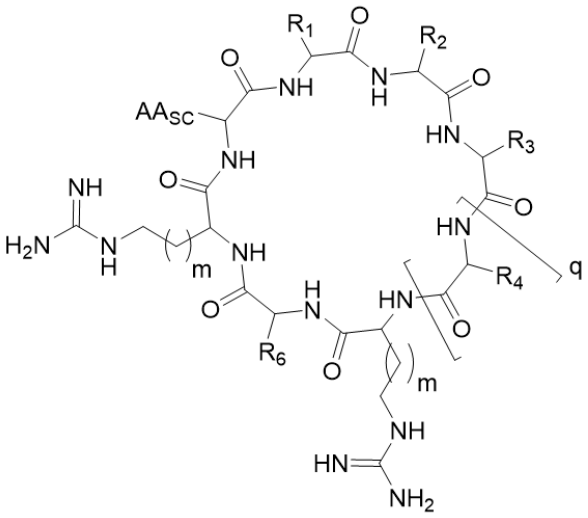
low and there remains a need for improved delivery systems to increase the potency of these antisense compounds.

[0004] There is an unmet need for effective compositions to deliver antisense compounds to intracellular compartments to treat diseases caused by, e.g., aberrant gene transcription, splicing and/or translation.

SUMMARY

[0004a] 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 44 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).

[0005] Compounds for delivering nucleic acids are described herein. In examples, the nucleic acids are antisense compounds (AC). In examples, the antisense compounds target exon 44 in a subject with Duchenne muscular dystrophy (DMD).

[0006] The disclosure relates to compounds comprising:

(a) a cell penetrating peptide (CPP) sequence (e.g., cyclic peptide); and

(b) an antisense compound (AC) that is complementary to a target sequence comprising at least a portion of exon 44 of *DMD* gene in a pre-mRNA sequence. In examples, the AC is complementary to a target sequence comprising at least a portion of exon 44 of *DMD* gene in a pre-mRNA sequence, at least a portion of an intronic sequence flanking exon 44 of *DMD* gene in a pre-mRNA sequence, or both. In examples, hybridization of the AC with the target sequence alters the splicing pattern of the *DMD* pre-mRNA to restore the reading frame and enable production of a functional dystrophin protein.

[0007] 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.

[0008] In examples, the AC comprises the sequence:

5'-AAA CGC CGC CAT TTC TCA ACA GAT C-3'.

[0009] In examples, the cyclic peptide is FGFGRGRQ. In examples, the cyclic peptide is GfFGrGrQ. In examples, the cyclic peptide is FfΦGRGRQ.

[0010] In examples, the EEV is: Ac-PKKKRKV-AEEA-Lys-(cyclo[FGFGRGRQ])-PEG12-OH.

[0011] The disclosure relates to a pharmaceutical composition comprising a compound described herein.

[0012] The disclosure relates to a cell comprising a compound described herein.

[0013] The disclosure relates to a method of treating DMD comprising administering a compound described herein to a patient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] **FIGs. 1A** and **1B** shows the conjugation chemistry for connecting a therapeutic moiety, e.g., an antisense compound (AC), to a cell penetrating peptide (CPP). The CPP can be conjugated to the 5' end, the 3' end or the backbone of the AC.

[0015] **FIGs. 2A and 2B** show conjugation chemistries 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.

[0016] **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.

[0017] **FIG. 4A** shows a schematic of preparation of EEV-PMO-MDX-23-1. **FIG. 4B** is a RT-PCR analysis that shows that, in comparison to mice treated with PMO-MDX-23-1, mice treated with EEV-PMO-MDX-23-1 produced dystrophin lacking the internal exon, exon 23. **FIG.4C** shows dystrophin exon skipping products in various treated muscle groups after administration of PMO-MDX-23-1 and EEV-PMO-MDX-23-1.

[0018] **FIGS. 5A-5D** show the percentage of exon skipping in MDX mice in the quadriceps (**FIG. 5A**), tibialis anterior (TA) (**FIG. 5B**), diaphragm (**FIG. 5C**), and heart (**FIG. 5D**) after delivery of PMO-MDX-23-1 or EEV-PMO-MDX-23-1.

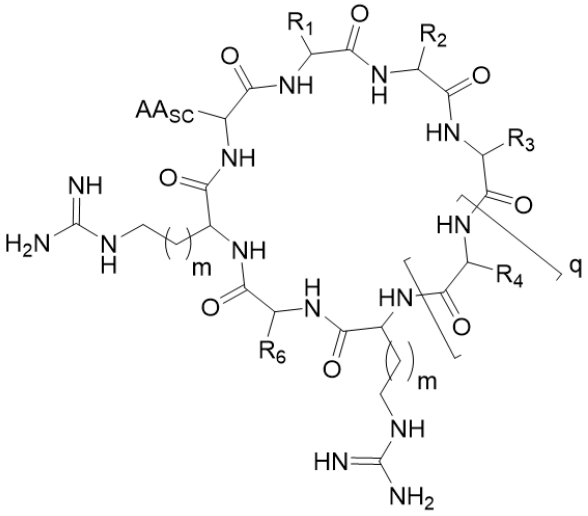
[0019] **FIGS. 6A-6D** show the percentage of exon 23 splicing in MDX mice in the tibialis anterior (TA) (**FIG. 6A**), quadriceps (**FIG. 6B**), diaphragm (**FIG. 6C**), and heart (**FIG. 6D**) after delivery of EEV-PMO-MDX-23-1.

CLAIMS

What is claimed is:

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 44 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 a phosphorodiamidate morpholino nucleotide (PMO) comprising 15-30 nucleotides.

3. 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.

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

5. 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’-XGAAAACGCCGCCAXXXCXCAACAGAXCXG-3’, wherein X is U or T, or a portion thereof.

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’- AAA CGC CGC CAT TTC TCA ACA GAT C-3’ or 5’-T GTT CAG CTT CTG TTA GCC ACT G AT-3’.

7. The compound of claim 6, wherein the AC comprises a sequence comprising at least 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 contiguous nucleotides of a base sequence comprising: 5’- AAA CGC CGC CAT TTC TCA ACA GAT C-3’ or 5’-T GTT CAG CTT CTG TTA GCC ACT G AT-3’.

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

a) 5’-GGCCAAACCTCGGCTTACTG AAAT-3’;

b) 5’-GGCCAAACCTCGGCTTACCTGAAAT-3’;

c) 5’-TGAAAACGCCGCCATTTCTCAACAGATCTG-3’;

d) 5’-TGAAAACGCCGCCATTTCTCAACAG-3’;

e) 5’-AAACGCCGCCATTTCTCAACAGATC-3’;

f) 5’-AACGCCGCCATTTCTCAACAGATCT-3’;

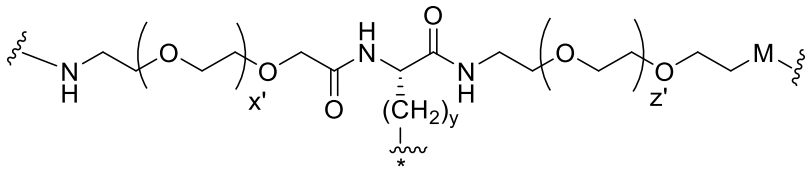
g) 5’-CCGCCATTTCTCAACAGATCTGTCA-3’;

h) 5’-TGTTCAGCTTCTGTTAGCCACTGAT-3’;

i) 5’-ACTGTTCAGCTTCTGTTAGCCACTG-3’; or

any reverse complement thereof.

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



, wherein

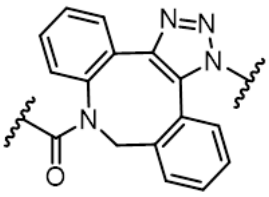
x’ is an integer from 1-23;

y is an integer from 1-5;

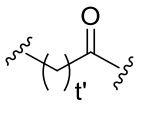
z’ is an integer from 1-23;

*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 1, wherein R_4 and R_6 are independently an amino acid side chain of citrulline or serine.

12. The compound of claim 1 wherein:

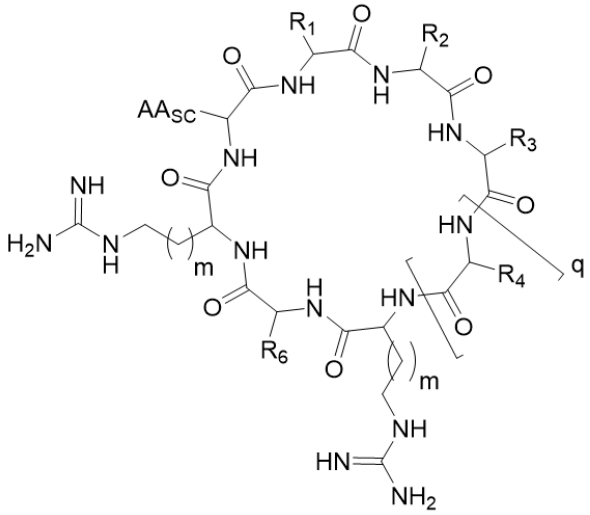
(i) the exocyclic peptide comprises 1 or 2 amino acid residues comprising a side chain comprising a guanidine group, or a protonated form thereof;

(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.

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

14. 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;

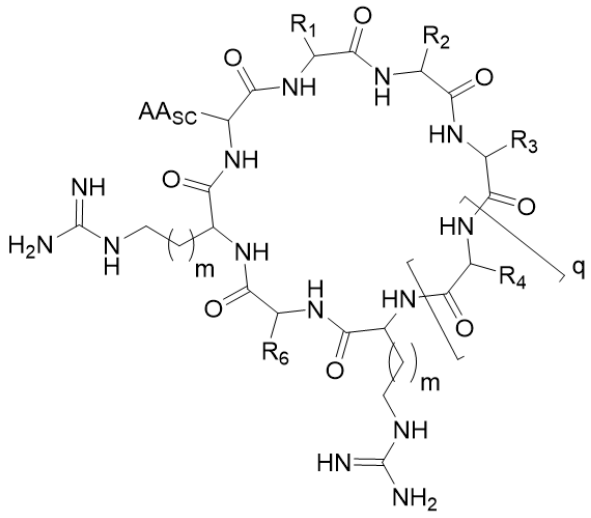
R_4 and R_6 are independently H or an amino acid side chain;

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.

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



, or a protonated form thereof,

wherein,

R_1, R_2, and R_3 are $-CH_2Ph$;

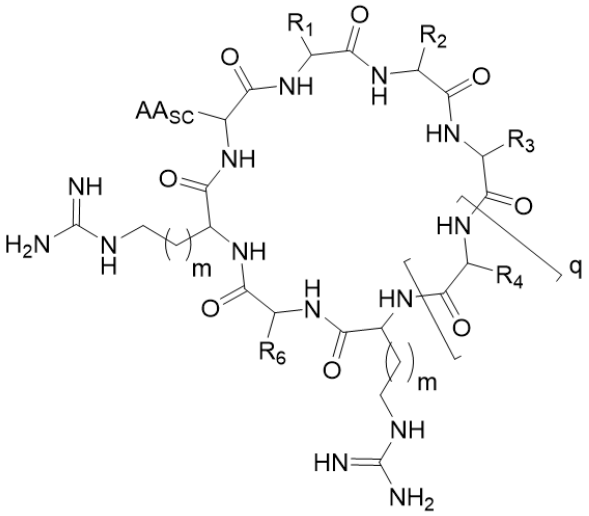
R_4 and R_6 are independently H or an amino acid side chain;

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.

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 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.

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

18. 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.

19. 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.

20. 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$_2$;

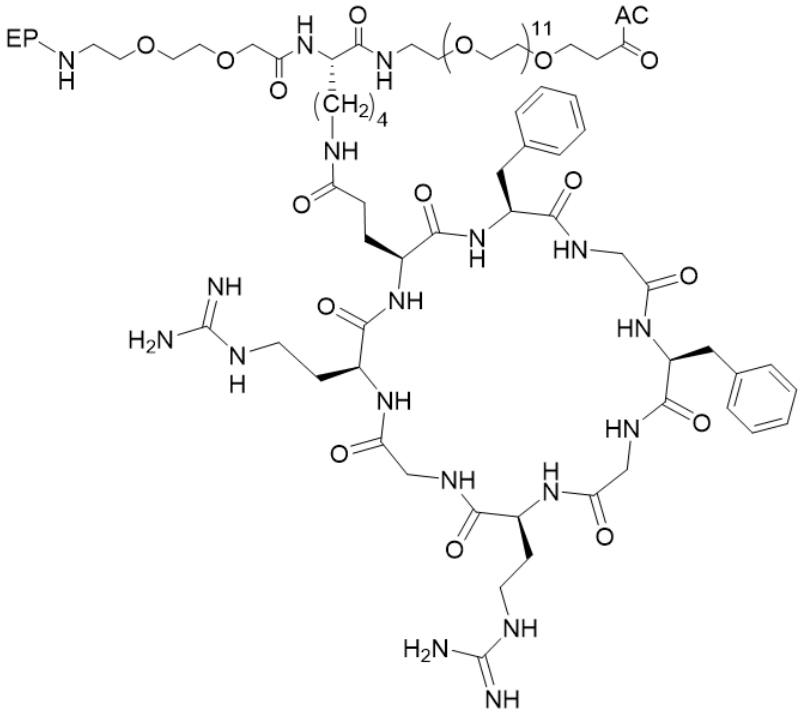
Ac-PKKKRKV-PEG$_2$-K(*cyclo*[FfFGRGRQ])-PEG$_2$-K(N3)-NH$_2$;

Ac-PKKKRKV-K(*cyclo*([Ff-Nal-GrGrQ])-PEG$_{12}$-K(N$_3$)-NH$_2$; or

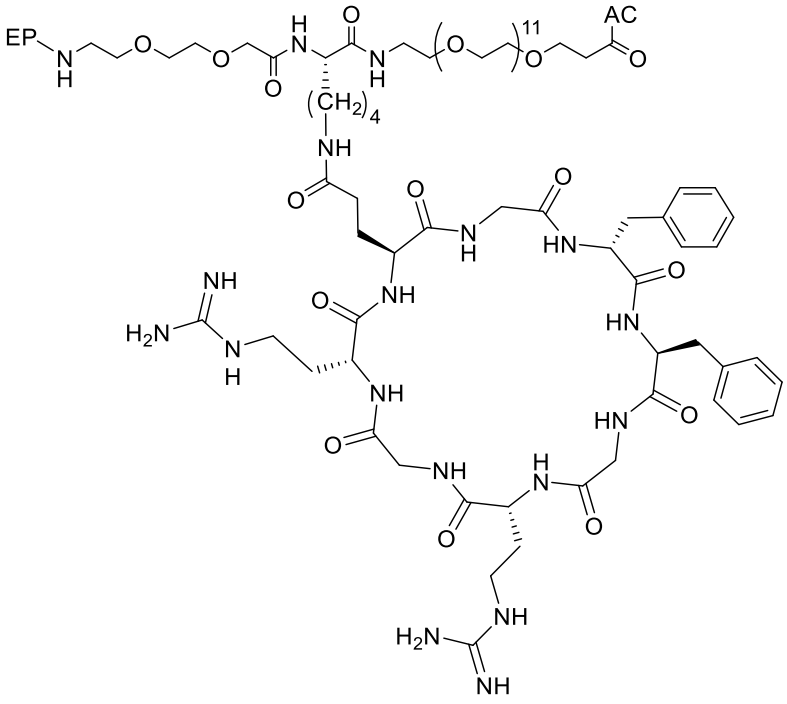
Ac-PKKKRKV-PEG$_2$-K(*cyclo*[Ff-Nal-GrGrQ])-PEG$_{12}$-OH;

prior to conjugation to the therapeutic moiety.

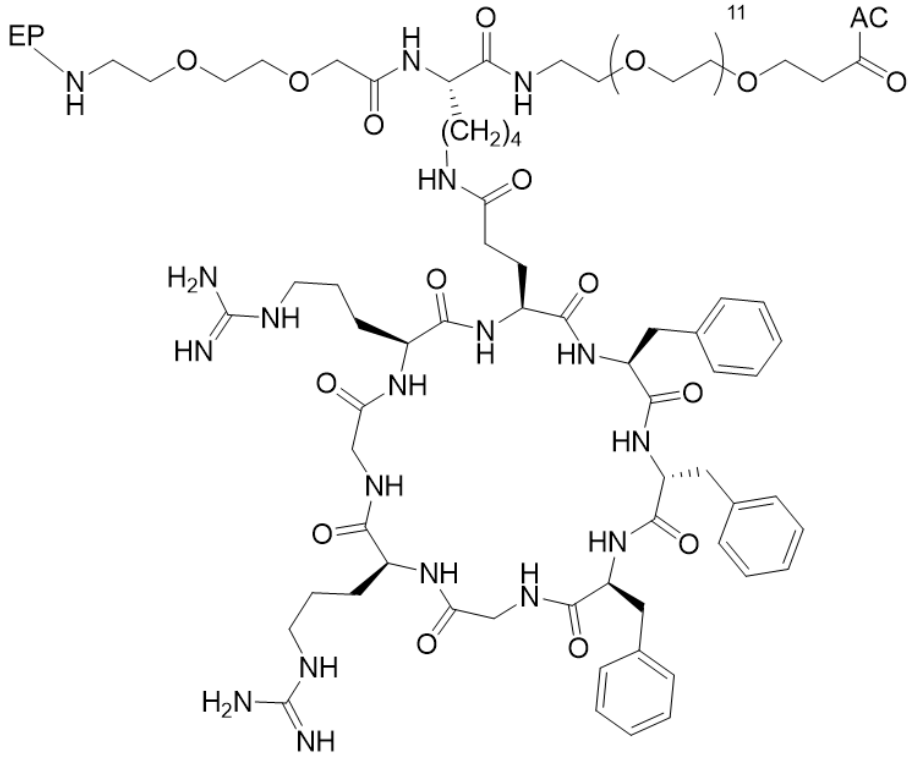
21. The compound of any one of claims 1 to 8, 12 or 13, wherein the Endosomal Escape Vehicle (EEV) conjugated to a therapeutic moiety comprising an antisense compound (AC) comprises the structure of Formula (C-1), Formula (C-2), Formula (C-3), or Formula (C-4):



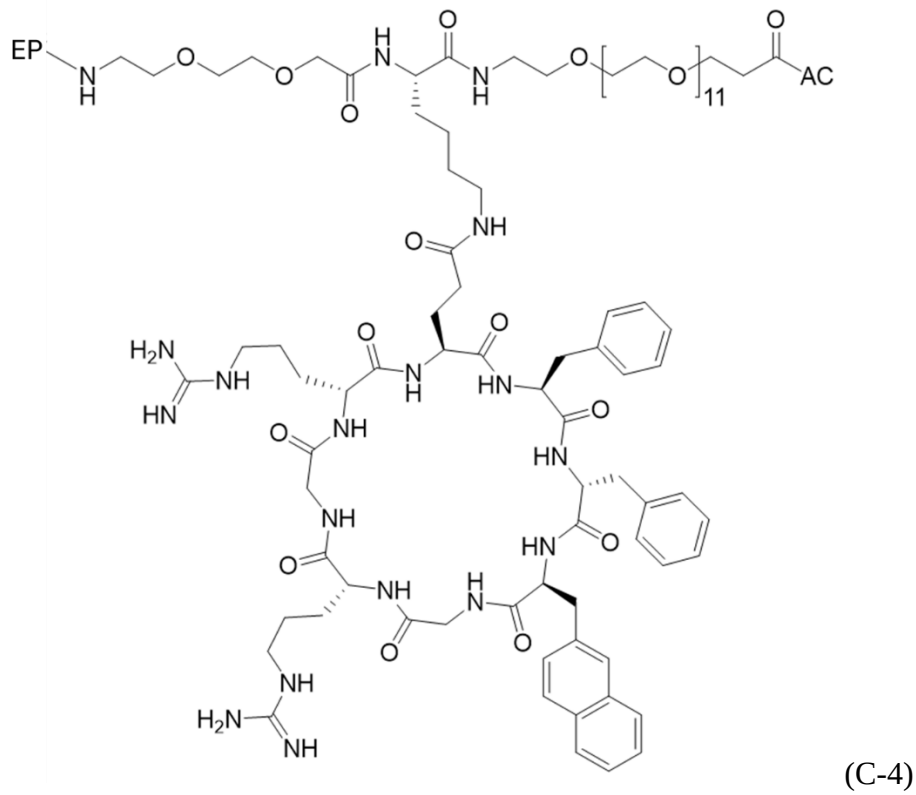
(C-1),



(C-2),



(C-3),



(C-4)

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