

IONIZABLE LIPIDS AND COMPOSITIONS FOR NUCLEIC ACID DELIVERY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to Chinese Patent Application No. 202110396368.4, filed April 13, 2021. The entire contents of the aforementioned application are incorporated herein by reference.

TECHNICAL FIELD

The present disclosure relates to ionizable lipids and its compositions for nucleic acid delivery, belonging to the field of medicinal chemistry.

BACKGROUND

Nucleic acid drugs include DNA, antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), microRNAs (miRNAs), miRNA mimics, antimiRs, ribozymes, mRNAs, aptamers, plasmids, and CRISPR RNAs. The application of nucleic acid drugs is limited by its chemical properties, which are easily degraded into a single nucleotide by nucleases *in vitro* and *in vivo*, resulting loss of activity.

Therefore, the application of nucleic acid drugs commonly requires special delivery vectors, including viral vectors and non-viral vectors. Viral vectors (including retroviruses, lentiviruses, adeno-associated viruses, etc.) have potent transfection efficiency. However, unfavorable immunogenicity, restricted loading capacity, complex production process and other factors limit its clinical application. Currently, non-viral vectors are a class of gene delivery vectors with good application prospects, which load mRNA by adsorption of cations formed by delivery materials with mRNA phosphate ions to form liposomes or nanoparticles, which protect them from nuclease degradation. Collectively, non-viral vectors is relatively easy to obtain, low immunogenicity, and high safety.

Traditionally, non-viral nucleic acid delivery materials are easily adsorbed by plasma proteins *in vivo* and then taken up by the reticuloendothelial system, resulting the loaded nucleic acid drugs are destroyed due to its strong positive electrical properties. Ionizable lipid-based nanoparticles were prepared by ionizable lipid-based materials, which realize the loading of nucleic acid drugs by electrostatic adsorption of nucleic acids and show positive

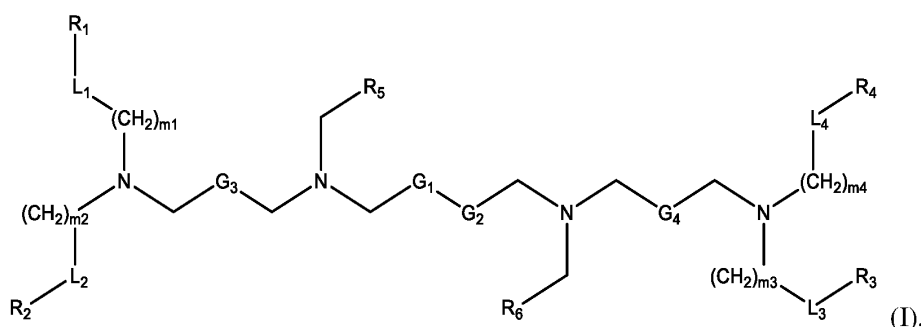
electricity in acidic environment *in vitro*. Importantly, they show electroneutrality to avoid the adsorption of plasma proteins and the capture of reticuloendothelial system after entering the neutral environment *in vivo*. Overall, ionizable nanoparticles have a very broad prospect in the field of nucleic acid delivery.

However, there are still relatively few clinical applications of ionizable nanoparticles. Therefore, the development of ionizable nucleic acid delivery materials with high efficiency and safety is of great significance for the wide application of nucleic acid drug gene therapy.

SUMMARY

Described herein are compounds of Formula (I) and pharmaceutically acceptable salts thereof that can be used as ionizable lipids for forming nucleic acid-lipid particles. It is unexpected to find that the ionizable lipids disclosed herein and the corresponding and nanoparticles have good encapsulation efficiency for mRNA. They also have stronger transfection ability, *in vivo* mRNA expression, immune anti-tumor effects.

In one aspect, the present disclosure provides a compound of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein the variables shown in the formula are defined herein.

Also provided are pharmaceutical compositions comprising a compound of Formula (I), a pharmaceutically acceptable salt thereof and a nucleic acid drug.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 II-1. Gel block results of LNP@mRNA.

Figure 2 shows Particle size, PDI and potential of II-1 LNP@mRNA.

Figure 3 shows photographs of microstructures of II-1, II-5, II-5, VI-1 LNP@Luc mRNA.

Figure 4 illustrates transfection rate of MC3, II-13, III-9, IV-4 LNP@GFP mRNA on DC2.4 cells.

Figure 5 shows expression and distribution of II-11, III-6, V-2 and MC3 LNP@Luc mRNA *in vivo*.

Figure 6 shows immunological anti-tumor effects of II-9, III-8, II-22, VI-4 LNP@OVA mRNA.

Figure 7 shows intramuscular injection safety effects of MC3, III-3, and VI-2 LNP@OVA mRNA.

Figure 8 shows *in vivo* expression of representative LNP@Luc mRNA via intramuscular injection.

Figure 9 shows Titers of RBD-specific IgG in the sera of immunized mice.

Figure 10 shows alanine transaminase (ALT) level of the immunized mice.

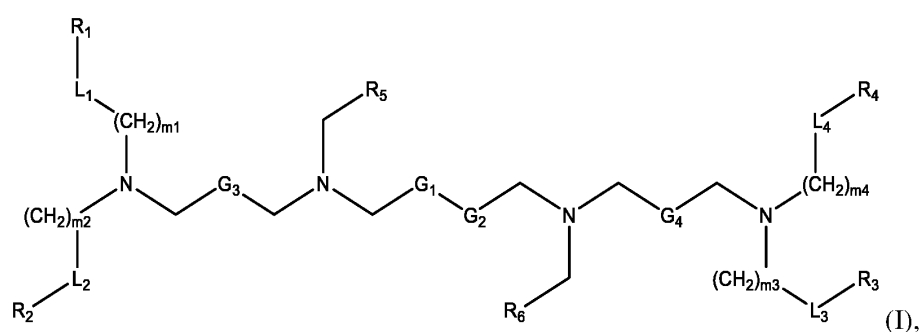
Figure 11 shows aspartate aminotransferase (AST) level of the immunized mice.

Figure 12 shows creatinine (CRE) level of the immunized mice.

DETAILED DESCRIPTION

The present disclosure aims to solve at least one of the existing technical problems and providing ionizable lipids for nucleic acid delivery.

In the first embodiment, the present disclosure provides a compound of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein

m1, m2, m3, and m4 are each independently selected from 1, 2, 3, 4, or 5;

L1, L2, L3, and L4 are each independently selected from -CH(OH)-, -C(=O)-, -C(=O)O-, -OC(=O)-, -C(=O)S-, -SC(=O)-, -C(=O)NR_a-, -NR_aC(=O)-, -NR_aC(=O)O-,

-OC(=O)NR_a-, -O-, -O-O-, -S-, -S-S-, -S-S-S-, -CH(OH)CH₂O-, -CH(OH)CH₂S-, or absent, wherein

each R_a is independently -H or optionally substituted C₁-C₆ alkyl;

R₁, R₂, R₃, and R₄ are each independently selected from optionally substituted C₆-C₃₀ alkyl, optionally substituted C₆-C₃₀ alkenyl, or optionally substituted C₆-C₃₀ alkynyl;

G₁, G₂, G₃ and G₄ are each independently selected from -R_c-, -R_cCH(OH)R_d-, -R_cC(=O)R_d-, -R_cC(=O)OR_d-, -R_cOC(=O)R_d-, -R_cC(=O)SR_d-, -R_cSC(=O)R_d-, -R_cC(=O)N(R_b)R_d-, -R_cN(R_b)C(=O)R_d-, -R_cN(R_b)C(=O)OR_d-, -R_cOC(=O)N(R_b)R_d-, -R_cOR_d-, -R_c-O-O-R_d-, -R_cSR_d-, -R_c-S-S-R_d-, -R_c-S-S-S-R_d-, or absent; wherein

each R_b is independently -H or optionally substituted C₁-C₆ alkyl;

each R_c and R_d are independently -(CH₂)_n-, and n is 0, 1, 2, 3, or 4;

R₅ and R₆ are each independently selected from -H, -OH, or optionally substituted C₁-C₆ alkyl.

In a second embodiment, the present disclosure provides a compound according to Formula (I), or a pharmaceutically acceptable salt thereof, wherein

G₁ and G₂ are each independently -R_c-, G₃ and G₄ are each independently selected from -R_c-, -R_cC(=O)R_d-, -R_cC(=O)OR_d-, -R_cOC(=O)R_d-, -R_cC(=O)N(R_b)R_d-, -R_cN(R_b)C(=O)R_d-, -R_cN(R_b)C(=O)OR_d-, -R_cOC(=O)N(R_b)R_d-, or absent;

each R_b is independently -H or C₁-C₆ alkyl;

each R_c and R_d are independently -(CH₂)_n-, and n is 0, 1, 2, 3, or 4. The definitions of the remaining variables are provided in the first embodiment.

In a third embodiment, the present disclosure provides a compound according to the second embodiment, or a pharmaceutically acceptable salt thereof, wherein

G₁ and G₂ are each independently -R_c-, G₃ and G₄ are each independently selected from -R_c-, -R_cC(=O)OR_d-, -R_cOC(=O)R_d-, -R_cC(=O)N(R_b)R_d-, -R_cN(R_b)C(=O)R_d-, or absent;

each R_b is independently -H or C₁-C₂ alkyl;

each R_c and R_d are independently -(CH₂)_n- or absent, and n is 0, 1 or 2. The definitions of the remaining variables are provided in the first or third embodiment.

In a fourth embodiment, the present disclosure provides a compound according to the third embodiment, or a pharmaceutically acceptable salt thereof, wherein G₁ and G₂ are absent, G₃ and G₄ are each independently selected from -CH₂-, -CH₂C(=O)OCH₂-, -CH₂OC(=O)CH₂-, -CH₂C(=O)NHCH₂-, -CH₂NHC(=O)CH₂-, or absent. The definitions of the remaining variables are provided in the first embodiment.

In a fifth embodiment, the present disclosure provides a compound according to the first embodiment, or a pharmaceutically acceptable salt thereof, wherein

G_3 and G_4 are each independently $-R_c-$, G_1 and G_2 are each independently selected from $-R_c-$, $-R_cC(=O)R_d-$, $-R_cC(=O)OR_d-$, $-R_cOC(=O)R_d-$, $-R_cC(=O)N(R_b)R_d-$, $-R_cN(R_b)C(=O)R_d-$, $-R_cN(R_b)C(=O)OR_d-$, $-R_cOC(=O)N(R_b)R_d-$, or absent;

each R_b is independently $-H$ or C_1 - C_6 alkyl;

each R_c and R_d are independently $-(CH_2)_n-$, and n is 0, 1, 2, 3, or 4. The definitions of the remaining variables are provided in the first embodiment.

In a sixth embodiment, the present disclosure provides a compound according to the fifth embodiment, or a pharmaceutically acceptable salt thereof, wherein

G_3 and G_4 are each independently $-R_c-$, G_1 and G_2 are each independently selected from $-R_c-$, $-R_cC(=O)OR_d-$, $-R_cOC(=O)R_d-$, $-R_cC(=O)N(R_b)R_d-$, $-R_cN(R_b)C(=O)R_d-$, or absent;

each R_b is independently $-H$ or C_1 - C_2 alkyl;

each R_c and R_d are independently $-(CH_2)_n-$ or absent, and n is 0, 1 or 2. The definitions of the remaining variables are provided in the first embodiment.

In a seventh embodiment, the present disclosure provides a compound according to the sixth embodiment, or a pharmaceutically acceptable salt thereof, wherein

G_3 and G_4 are absent, G_1 and G_2 are each independently selected from $-CH_2-$, $-CH_2C(=O)O(CH_2)_{1 \text{ or } 2}-$, $-(CH_2)_{1 \text{ or } 2}OC(=O)CH_2-$, $-CH_2C(=O)N(R_b)CH_2-$, $-CH_2N(R_b)C(=O)CH_2-$, or absent;

each R_b is independently $-H$ or C_1 - C_2 alkyl. The definitions of the remaining variables are provided in the first embodiment.

In an eighth embodiment, the present disclosure provides a compound according to any one of the first through seventh embodiments, or a pharmaceutically acceptable salt thereof, wherein L_1 , L_2 , L_3 , and L_4 are each independently selected from $-CH(OH)-$, $-C(=O)-$, $-C(=O)O-$, $-OC(=O)-$, $-C(=O)NR_a-$, $-NR_aC(=O)-$, $-NR_aC(=O)O-$, $-OC(=O)NR_a-$, $-O-$, $-S-$, $-CH(OH)CH_2O-$, $-CH(OH)CH_2S-$, or absent. The definitions of the remaining variables are provided in any one of the first through seventh embodiments.

In a ninth embodiment, the present disclosure provides a compound according to any one of the first through seventh embodiments, or a pharmaceutically acceptable salt thereof, wherein L_1 , L_2 , L_3 , and L_4 are each independently selected from $-CH(OH)-$, $-C(=O)-$, $-C(=O)O-$, $-OC(=O)-$, $-C(=O)NR_a-$, $-NR_aC(=O)-$, $-O-$, $-S-$, or absent; each R_a is

independently -H or C₁-C₂ alkyl. The definitions of the remaining variables are provided in any one of the first through seventh embodiments.

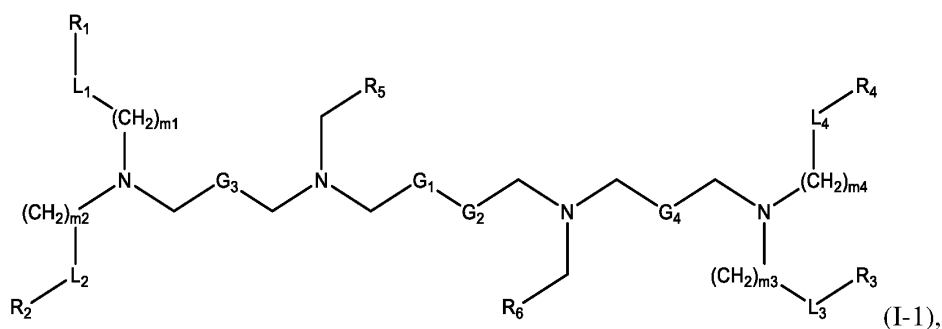
In a tenth embodiment, the present disclosure provides a compound according to any one of the first through ninth embodiments, or a pharmaceutically acceptable salt thereof, wherein m₁, m₂, m₃, and m₄ are each independently selected from 1 or 2. The definitions of the remaining variables are provided in any one of the first through ninth embodiments.

In an eleventh embodiment, the present disclosure provides a compound according to any one of the first through tenth embodiments, or a pharmaceutically acceptable salt thereof, wherein R₁, R₂, R₃, and R₄ are each independently selected from C₆-C₁₈ alkyl, C₆-C₁₈ alkenyl, or C₆-C₁₈ alkynyl, wherein said C₆-C₁₈ alkyl, C₆-C₁₈ alkenyl, or C₆-C₁₈ alkynyl is optionally substituted one to three groups selected from halogen, OH, or =O. The definitions of the remaining variables are provided in any one of the first through tenth embodiments.

In a twelfth embodiment, the present disclosure provides a compound according to any one of the first through tenth embodiments, or a pharmaceutically acceptable salt thereof, wherein R₁, R₂, R₃, and R₄ are each independently selected from C₆-C₁₈ alkyl. The definitions of the remaining variables are provided in any one of the first through tenth embodiments.

In a thirteenth embodiment, the present disclosure provides a compound according to any one of the first through twelfth embodiments, or a pharmaceutically acceptable salt thereof, wherein R₅ and R₆ are each independently selected from -H, -OH, or C₁-C₄ alkyl optionally substituted with -OH. The definitions of the remaining variables are provided in any one of the first through twelfth embodiments.

In a fourteenth embodiment, the present disclosure provides a compound of Formula (I-1):



or a pharmaceutically acceptable salt thereof, wherein

m₁, m₂, m₃, and m₄ are the same, all of which are 1, 2, 3, 4, or 5;

L₁, L₂, L₃, and L₄ are the same, all of which are selected from -CH(OH)-, -C(=O)-,

$\wedge$ -C(=O)O- $\wedge\wedge$, $\wedge$ -OC(=O)- $\wedge\wedge$, $\wedge$ -C(=O)S- $\wedge\wedge$, $\wedge$ -SC(=O)- $\wedge\wedge$, $\wedge$ -C(=O)NR_a- $\wedge\wedge$, $\wedge$ -NR_aC(=O)- $\wedge\wedge$,
 $\wedge$ -NR_aC(=O)O- $\wedge\wedge$, $\wedge$ -OC(=O)NR_a- $\wedge\wedge$, -O-, -O-O-, -S-, -S-S-, -S-S-S-, $\wedge$ -CH(OH)CH₂O- $\wedge\wedge$,
 $\wedge$ -CH(OH)CH₂S- $\wedge\wedge$ or absent, wherein

$\wedge$ - represents the point which attaches to R₁₋₄; - $\wedge\wedge$ represents the point which attaches to -(CH₂)_{m1-m4}-; and

each R_a is independently -H or optionally substituted C₁-C₆ alkyl;

R₁, R₂, R₃, and R₄ are the same, all of which are selected from optionally substituted C₆-C₃₀ alkyl, optionally substituted C₆-C₃₀ alkenyl, or optionally substituted C₆-C₃₀ alkynyl;

G₁ and G₂ are the same, and G₃ and G₄ are the same,

when G₁ and G₂ are -R_c-, G₃ and G₄ are selected from -R_c-, *-R_cCH(OH)R_d-**,
 *-R_cC(=O)R_d**, *-R_cC(=O)OR_d**, *-R_cOC(=O)R_d**, *-R_cC(=O)SR_d**,
 *-R_cSC(=O)R_d**, *-R_cC(=O)N(R_b)R_d**, *-R_cN(R_b)C(=O)R_d**, *-R_cN(R_b)C(=O)OR_d**,
 *-R_cOC(=O)N(R_b)R_d**, *-R_cOR_d**, *-R_c-O-O-R_d**, *-R_cSR_d**, *-R_c-S-S-R_d**,
 *-R_c-S-S-S-R_d**, or absent; wherein

*- represents the point which attaches to the -CH₂- group next to the terminal tertiary amine atom as shown in Formula (I); -** represents the point which attaches to the -CH₂- group next to the middle tertiary amine atom as shown in Formula (I);

when G₃ and G₄ are -R_c-, G₁ and G₂ are selected from -R_c-, #-R_cCH(OH)R_d-##,
 #-R_cC(=O)R_d-##, #-R_cC(=O)OR_d-##, #-R_cOC(=O)R_d-##, #-R_cC(=O)SR_d-##, #-R_cSC(=O)R_d-##,
 #-R_cC(=O)N(R_b)R_d-##, #-R_cN(R_b)C(=O)R_d-##, #-R_cN(R_b)C(=O)OR_d-##,
 #-R_cOC(=O)N(R_b)R_d-##, #-R_cOR_d-##, #-R_c-O-O-R_d-##, #-R_cSR_d-##, #-R_c-S-S-R_d-##,
 #-R_c-S-S-S-R_d-##, or absent;

wherein

#- represents the point which attaches to the -CH₂- group next to the middle tertiary amine atom as shown in Formula (I); -## represents the point connecting G₁ and G₂; and

each R_b is independently -H or optionally substituted C₁-C₆ alkyl;

each R_c and R_d are independently -(CH₂)_n-, and n is 0, 1, 2, 3, or 4;

R₅ and R₆ are the same, both of which are selected from -H, -OH, or optionally substituted C₁-C₆ alkyl.

The terminal tertiary amine atom described herein refers to the two nitrogen atoms which are connected with -(CH₂)_{m1-m4}-L₁₋₄-R₁₋₄ moieties. The middle tertiary amine atom described herein refers to the two nitrogen atoms which are connected with -CH₂R₅ or

-CH₂R₆.

In a fifteenth embodiment, the present disclosure provides a compound according to the fourteenth embodiment, or a pharmaceutically acceptable salt thereof, wherein

G₁ and G₂ are -R_c-, G₃ and G₄ are selected from -R_c-, *-R_cC(=O)R_d-**, *-R_cC(=O)OR_d-**, *-R_cOC(=O)R_d-**, *-R_cC(=O)N(R_b)R_d-**, *-R_cN(R_b)C(=O)R_d-**, *-R_cN(R_b)C(=O)OR_d-**, *-R_cOC(=O)N(R_b)R_d-**, or absent;

each R_b is independently -H or C₁-C₆ alkyl;

each R_c and R_d are independently -(CH₂)_n-, and n is 0, 1, 2, 3, or 4. The definitions of the remaining variables are provided in the fourteenth embodiment.

In a sixteenth embodiment, the present disclosure provides a compound according to the fifteenth embodiment, or a pharmaceutically acceptable salt thereof, wherein

G₁ and G₂ are -R_c-, G₃ and G₄ are selected from *-R_cC(=O)OR_d-**, *-R_cOC(=O)R_d-**, *-R_cC(=O)N(R_b)R_d-**, *-R_cN(R_b)C(=O)R_d-**, or absent;

each R_b is independently -H or C₁-C₂ alkyl;

each R_c and R_d are independently -(CH₂)_n- or absent, and n is 0, 1 or 2. The definitions of the remaining variables are provided in the fourteenth embodiment.

In a seventeenth embodiment, the present disclosure provides a compound according to the sixteenth embodiment, or a pharmaceutically acceptable salt thereof, wherein G₁ and G₂ are absent, G₃ and G₄ are selected from -CH₂-, *-CH₂C(=O)OCH₂-**, *-CH₂OC(=O)CH₂-**, *-CH₂C(=O)NHCH₂-**, *-CH₂NHC(=O)CH₂-**, or absent. The definitions of the remaining variables are provided in the fourteenth or sixteenth embodiment.

In an eighteenth embodiment, the present disclosure provides a compound according to the fourteenth embodiment, or a pharmaceutically acceptable salt thereof, wherein

G₃ and G₄ are -R_c-, G₁ and G₂ are selected from -R_c-, #-R_cC(=O)R_d-##, #-R_cC(=O)OR_d-##, #-R_cOC(=O)R_d-##, #-R_cC(=O)N(R_b)R_d-##, #-R_cN(R_b)C(=O)R_d-##, #-R_cN(R_b)C(=O)OR_d-##, #-R_cOC(=O)N(R_b)R_d-##, or absent;

each R_b is independently -H or C₁-C₆ alkyl;

each R_c and R_d are independently -(CH₂)_n-, and n is 0, 1, 2, 3, or 4. The definitions of the remaining variables are provided in the fourteenth embodiment.

In a nineteenth embodiment, the present disclosure provides a compound according to the eighteenth embodiment, or a pharmaceutically acceptable salt thereof, wherein

G₃ and G₄ are -R_c-, G₁ and G₂ are selected from -R_c-, #-R_cC(=O)OR_d-##, #-R_cOC(=O)R_d-##, #-R_cC(=O)N(R_b)R_d-##, #-R_cN(R_b)C(=O)R_d-##, or absent;

each R_b is independently $-H$ or C_1 - C_2 alkyl;

each R_c and R_d are independently $-(CH_2)_n-$ or absent, and n is 0, 1 or 2. The definitions of the remaining variables are provided in the fourteenth embodiment.

In an twentieth embodiment, the present disclosure provides a compound according to the nineteenth embodiment, or a pharmaceutically acceptable salt thereof, wherein G_3 and G_4 are absent, G_1 and G_2 are selected from $-CH_2-$, $^{\#}-CH_2C(=O)O(CH_2)_{1 \text{ or } 2}-^{\#\#}$, $^{\#}-(CH_2)_{1 \text{ or } 2}OC(=O)CH_2-^{\#\#}$, $^{\#}-CH_2C(=O)N(R_b)CH_2-^{\#\#}$, $^{\#}-CH_2N(R_b)C(=O)CH_2-^{\#\#}$, or absent; each R_b is independently $-H$ or C_1 - C_2 alkyl. The definitions of the remaining variables are provided in the fourteenth embodiment.

In a twenty-first embodiment, the present disclosure provides a compound according to any one of the fourteenth through twentieth embodiments, or a pharmaceutically acceptable salt thereof, wherein L_1 , L_2 , L_3 , and L_4 are the same, all of which are selected from $-CH(OH)-$, $-C(=O)-$, $^{\wedge}-C(=O)O-^{\wedge\wedge}$, $^{\wedge}-OC(=O)-^{\wedge\wedge}$, $^{\wedge}-C(=O)NR_a-^{\wedge\wedge}$, $^{\wedge}-NR_aC(=O)-^{\wedge\wedge}$, $^{\wedge}-NR_aC(=O)O-^{\wedge\wedge}$, $^{\wedge}-OC(=O)NR_a-^{\wedge\wedge}$, $-O-$, $-S-$, $^{\wedge}-CH(OH)CH_2O-^{\wedge\wedge}$, $^{\wedge}-CH(OH)CH_2S-^{\wedge\wedge}$, or absent. The definitions of the remaining variables are provided in any one of the fourteenth through twentieth embodiments.

In a twenty-second embodiment, the present disclosure provides a compound according to any one of the fourteenth through twentieth embodiments, or a pharmaceutically acceptable salt thereof, wherein L_1 , L_2 , L_3 , and L_4 are the same, all of which are selected from $-CH(OH)-$, $-C(=O)-$, $^{\wedge}-C(=O)O-^{\wedge\wedge}$, $^{\wedge}-OC(=O)-^{\wedge\wedge}$, $^{\wedge}-C(=O)NR_a-^{\wedge\wedge}$, $^{\wedge}-NR_aC(=O)-^{\wedge\wedge}$, $-O-$, $-S-$, or absent; each R_a is independently $-H$ or C_1 - C_2 alkyl. The definitions of the remaining variables are provided in any one of the fourteenth through twentieth embodiments.

In a twenty-third embodiment, the present disclosure provides a compound according to any one of the fourteenth through twenty-second embodiments, or a pharmaceutically acceptable salt thereof, wherein m_1 , m_2 , m_3 , and m_4 are the same, all of which are 1 or 2. The definitions of the remaining variables are provided in any one of the fourteenth through twenty-second embodiments.

In a twenty-fourth embodiment, the present disclosure provides a compound according to any one of the fourteenth through twenty-third embodiments, or a pharmaceutically acceptable salt thereof, wherein R_1 , R_2 , R_3 , and R_4 are the same, all of which are selected from C_6 - C_{18} alkyl, C_6 - C_{18} alkenyl, or C_6 - C_{18} alkynyl, wherein said C_6 - C_{18} alkyl, C_6 - C_{18} alkenyl, or C_6 - C_{18} alkynyl is optionally substituted one to three groups

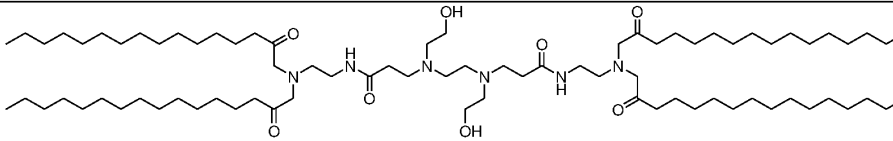
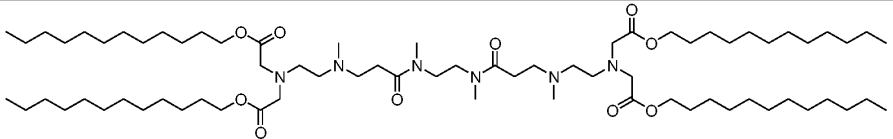
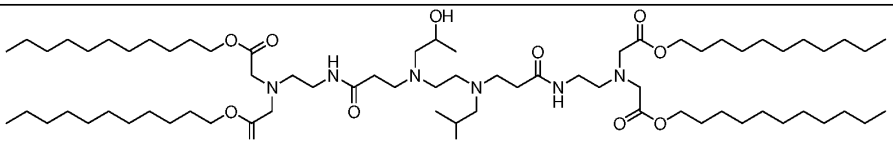
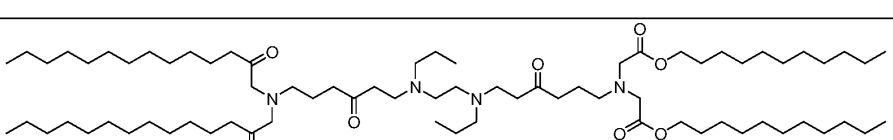
selected from halogen, OH, or =O. The definitions of the remaining variables are provided in any one of the fourteenth through twenty-third embodiments.

In a twenty-fifth embodiment, the present disclosure provides a compound according to any one of the fourteenth through twenty-third embodiments, or a pharmaceutically acceptable salt thereof, wherein R₁, R₂, R₃, and R₄ are the same, all of which are selected from C₆-C₁₈ alkyl. The definitions of the remaining variables are provided in any one of the fourteenth through twenty-third embodiments.

In a twenty-sixth embodiment, the present disclosure provides a compound according to any one of the fourteenth through twenty-fifth embodiments, or a pharmaceutically acceptable salt thereof, wherein R₅ and R₆ are the same, both of which are selected from -H, -OH, or C₁-C₄ alkyl optionally substituted with -OH. The definitions of the remaining variables are provided in any one of the fourteenth through twenty-fifth embodiments.

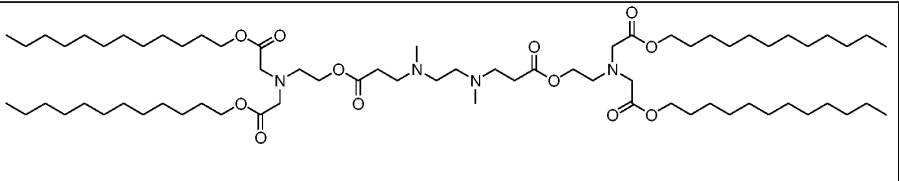
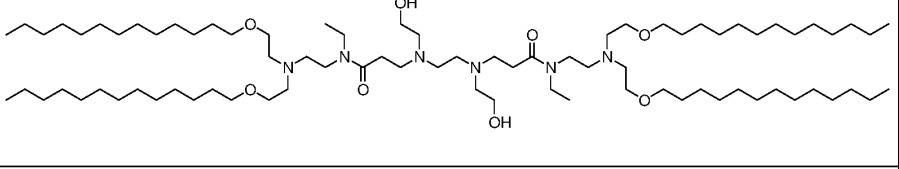
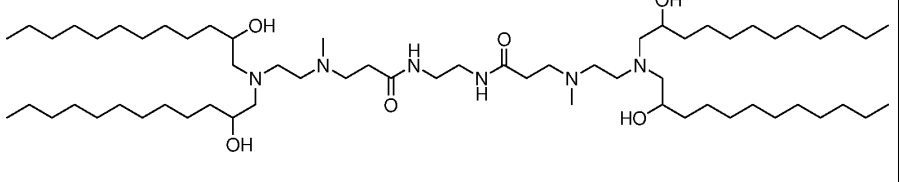
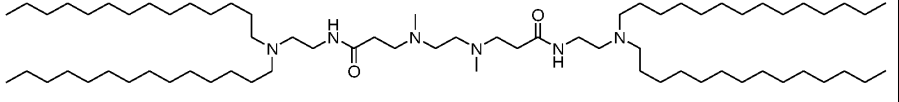
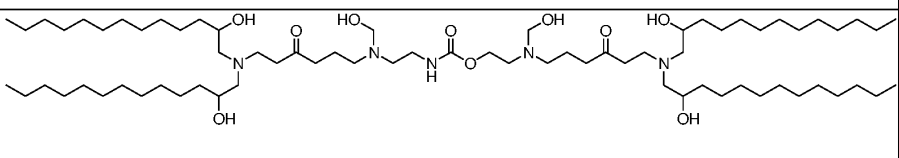
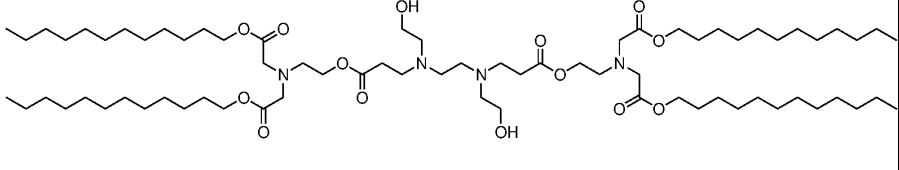
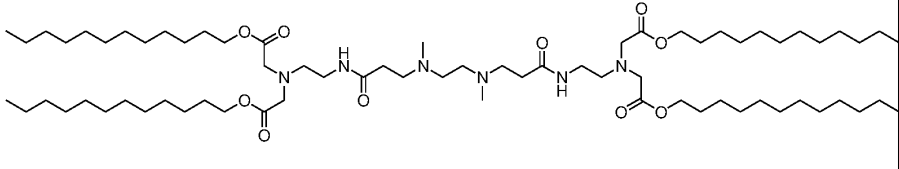
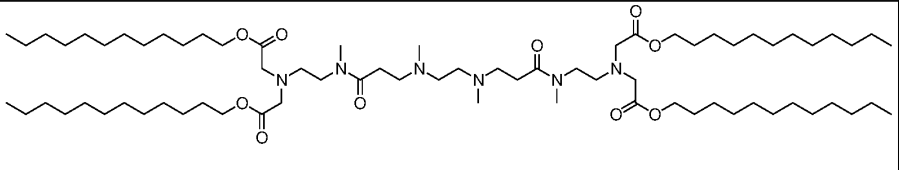
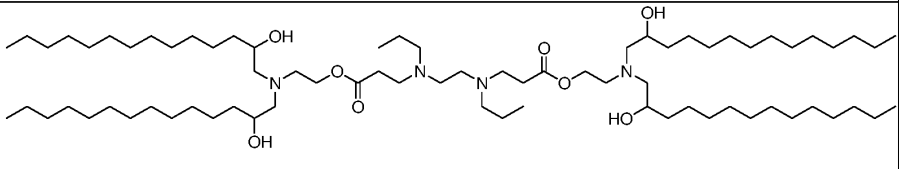
In one embodiment, the present disclosure provides a compound selected from the compounds disclosed in examples and Table 1, or a pharmaceutically acceptable salt thereof.

Table 1

Number	Code	Chemical formula
1	II-1	
2	III-1	
3	II-2	
4	II-3	

5	II-4	
6	III-2	
7	V-1	
8	II-5	
9	III-3	
10	IV-1	
11	VI-1	
12	II-6	
13	IV-2	

14	II-7	
15	VI-2	
16	II-8	
17	II-9	
18	II-10	
19	III-4	
20	VI-3	
21	III-5	

22	II-11	
23	II-12	
24	III-6	
25	II-13	
26	IV-3	
27	II-14	
28	II-15	
29	II-16	
30	II-17	

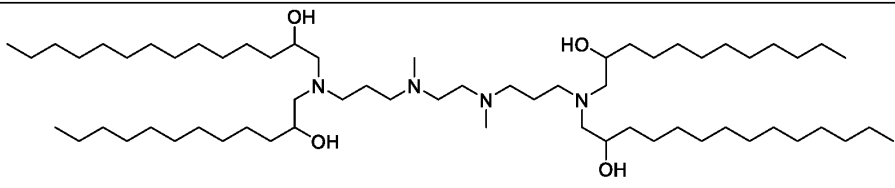
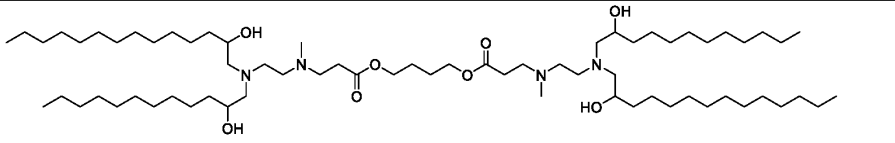
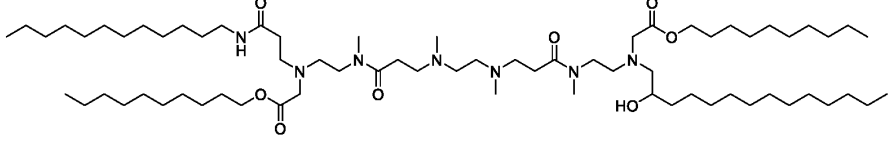
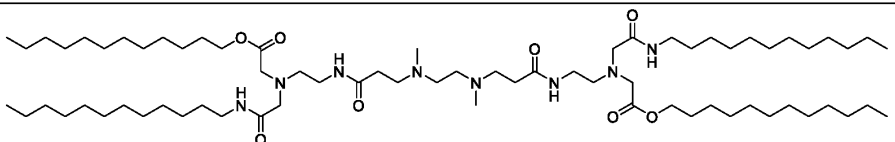
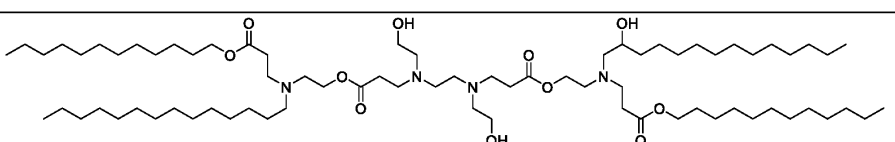
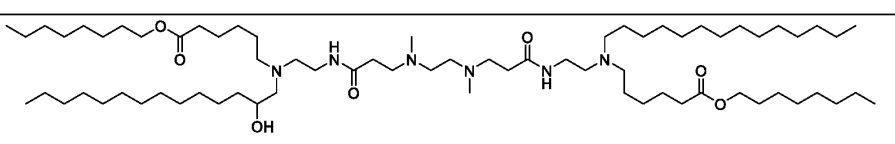
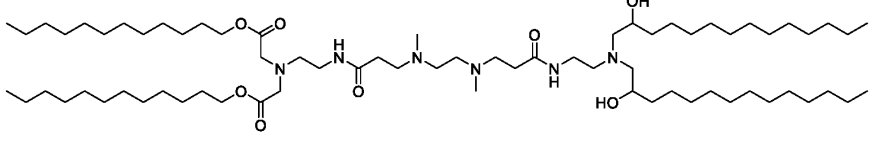
31	II-18	
32	II-19	
33	V-2	
34	III-7	
35	II-20	
36	III-8	
37	II-21	
38	V-3	
39	IV-4	

40	II-22	
41	III-9	
42	II-23	
43	III-10	
44	V-4	
45	VI-4	
46	II-24	
47	II-25	

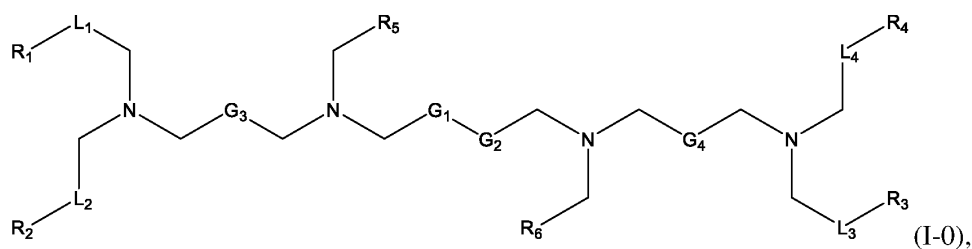
48	II-26	
49	III-11	
50	III-12	
51	II-27	
52	II-28	
53	III-13	
54	III-14	
55	II-29	

56	II-30	
57	II-31	
58	II-32	
59	III-15	
60	II-33	
61	II-34	
62	II-35	
63	II-36	

64	III-16	
65	II-37	
66	II-38	
67	VII-1	
68	VII-2	
69	VII-3	
70	VII-4	
71	VII-5	
72	VII-6	

73	VII-7	
74	VII-8	
75	VII-9	
76	VII-10	
77	VII-11	
78	VII-12	
79	VII-13	

The present disclosure also provides compounds as shown in Formula (I-0), or pharmaceutically acceptable salts, isomers, deuterated substitutes or prodrugs thereof;



wherein L_1, L_2, L_3, L_4 are independently selected from $-R_kCH(OH)-$, $-R_kC(=O)-$, $-R_kC(=O)O-$, $-R_kOC(=O)-$, $-R_kC(=O)S-$, $-R_kSC(=O)-$, $-R_kC(=O)NR_a-$, $-R_kNR_aC(=O)-$, $-R_kNR_aC(=O)O-$, $-R_kOC(=O)NR_a-$, $-R_kO-$, $-R_k-O-O-$, $-R_kS-$, $-R_k-S-S-$, $-R_k-S-S-S-$, $-R_kCH(OH)CH_2O-$, $-R_kCH(OH)CH_2S-$ or missing, R_k is $-(CH_2)_k-$ or missing, k is an integer above 1, and R_a is a $-H$, substituted or unsubstituted alkyl group;

R_1, R_2, R_3, R_4 are independently selected from C_1 - C_{30} linear alkyl, C_1 - C_{30} branched alkyl, C_2 - C_{30} linear alkenyl, C_2 - C_{30} branched enyl, C_2 - C_{30} linear ethinyl, or C_2 - C_{30} branched ethinyl;

G_1, G_2, G_3, G_4 are independently selected from $-R_c-$, $-R_cCH(OH)R_d-$, $-R_cC(=O)R_d-$, $-R_cC(=O)OR_d-$, $-R_cOC(=O)R_d-$, $-R_cC(=O)SR_d-$, $-R_cSC(=O)R_d-$, $-R_cC(=O)N(R_b)R_d-$, $-R_cN(R_b)C(=O)R_d-$, $-R_cN(R_b)C(=O)OR_d-$, $-R_cOC(=O)N(R_b)R_d-$, $-R_cOR_d-$, $-R_c-O-O-R_d-$, $-R_cSR_d-$, $-R_c-S-S-R_d-$, $-R_c-S-S-S-R_d-$ or absent, R_b is $-H$, substituted or unsubstituted alkyl groups, R_c, R_d are independently selected from $-(CH_2)_n-$ or do not exist, and n is an integer above 1;

R_5, R_6 are independently selected from $-OH$, $-H$, substituted or unsubstituted alkyl group.

Further, k is an integer of 1 ~ 10.

Further, k is 1.

Further, L_1, L_2, L_3, L_4 are independently selected from $-CH(OH)-$, $-C(=O)-$, $-CH_2C(=O)O-$, $-C(=O)O-$, $-OC(=O)-$, $-C(=O)S-$, $-SC(=O)-$, $-CH_2C(=O)NR_a-$, $-C(=O)NR_a-$, $-NR_aC(=O)-$, $-NR_aC(=O)O-$, $-OC(=O)NR_a-$, $-CH_2O-$, $-O-$, $-CH_2-O-O-$, $-CH_2S-$, $-S-$, $-CH_2-S-S-$, $-CH(OH)CH_2O-$, $-CH(OH)CH_2S-$ or absent, and R_a is a $-H$, substituted or unsubstituted alkyl group.

Further, L_1, L_2, L_3, L_4 are independently selected from $-C(=O)-$, $-C(=O)NR_a-$, $-CH_2C(=O)NR_a-$, $-NR_aC(=O)-$, $-C(=O)O-$, $-CH_2C(=O)O-$, $-OC(=O)-$, $-CH_2O-$, $-O-$, $-CH_2S-$, $-CH(OH)-$, $-CH(OH)CH_2O-$, $-CH(OH)CH_2S-$ or absent, and R_a is a $-H$ or an unsubstituted alkyl group.

Further, R_a is a $-H$ or an unsubstituted C_1 ~ C_6 alkyl group.

Further, R_a is a -H.

Further, L_1 , L_2 , L_3 , L_4 are independently selected from -C(=O)-, -C(=O)NH-, -CH₂C(=O)NH-, -C(=O)O-, -CH₂C(=O)O-, -CH₂O-, -CH₂S-, -CH(OH)-, -CH(OH)CH₂O- or absent; preferably, L_1 , L_2 , L_3 , L_4 are independently selected from -C(=O)NH-, -C(=O)O-, -CH(OH)-, -CH(OH)CH₂O- or absent.

Further, L_1 and L_2 are selected from the same group, and L_3 and L_4 are selected from the same group.

Further, L_1 , L_2 , L_3 and L_4 are selected from the same group.

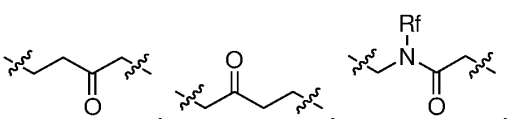
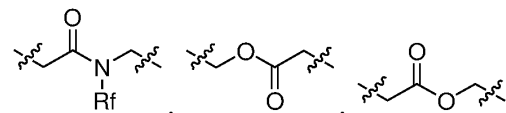
Further, R_1 , R_2 , R_3 , R_4 are independently selected from C₁-C₃₀ linear alkyl, C₂-C₃₀ linear alkenyl, C₂-C₃₀ linear ethinyl.

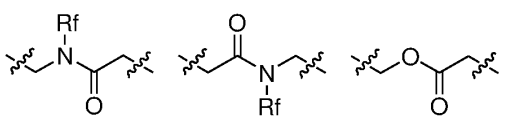
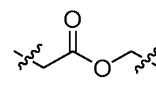
Further, R_1 , R_2 , R_3 , R_4 are independently selected from unsubstituted C₁-C₃₀ linear alkyl groups.

Further, R_1 , R_2 , R_3 and R_4 are independently selected from self-unsubstituted C₈ ~ C₁₈ linear alkyl groups.

Further, R_1 , R_2 , R_3 and R_4 are independently selected from unsubstituted C₁₀ ~ C₁₄ linear alkyl groups.

Further, R_1 , R_2 , R_3 and R_4 are selected from the same group.

Further, G₃ is selected from , , -(CH₂)_{n3}- or absent, R_f is a -H or an unsubstituted alkyl, and n_3 is an integer from 1 ~ 10.

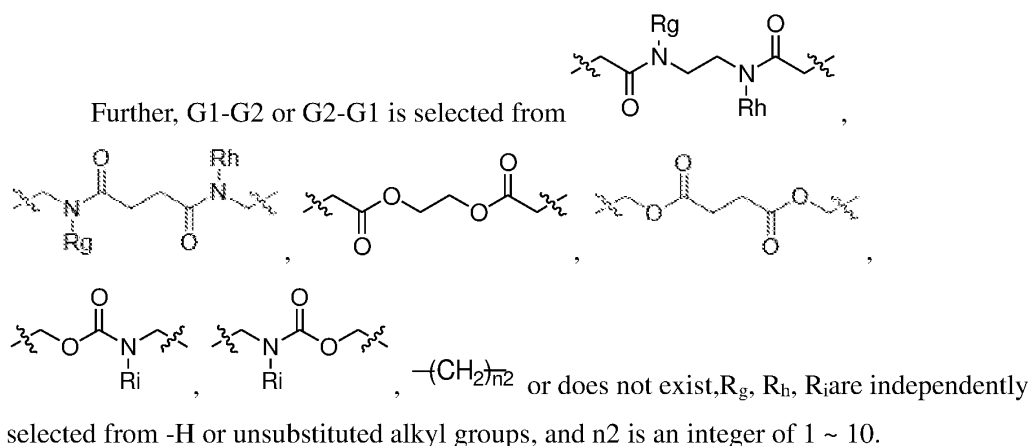
Preferably, G₃ is selected from , , -(CH₂)_{n3}- or absent, R_f is a -H or unsubstituted alkyl, and n_3 is an integer from 1 ~ 10.

Further, R_f is either a -H or an unsubstituted C₁ ~ C₆ alkyl group.

Further, R_f is a -H, methyl, ethyl, or propyl.

Further, n_3 is 1 or 2.

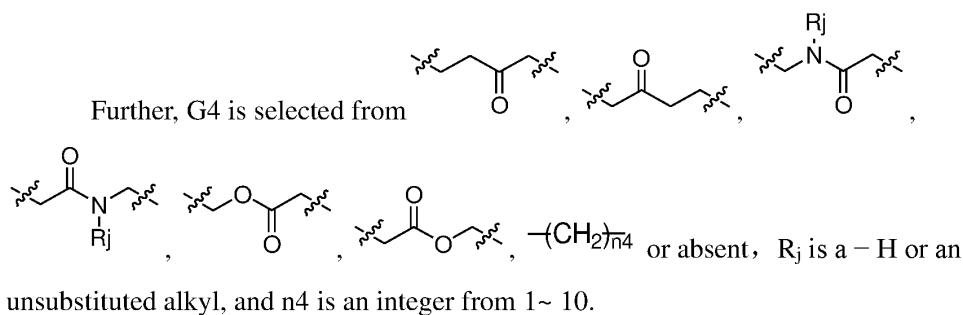
Preferably, n_3 is 1.



Further, R_g , R_h , and R_i are independently selected from -H or unsubstituted $C_1 \sim C_6$ alkyl groups.

Further, R_g , R_h , and R_i are independently selected from -H, methyl or ethyl groups.

Further, n_2 is 1 or 2.



Further, R_j is a -H or an unsubstituted $C_1 \sim C_6$ alkyl group.

Further, R_j is -H, methyl, ethyl, or propyl.

Further, n_4 is 1 or 2.

Further, R_5 , R_6 are independently selected from -OH, -H, unsubstituted $C_1 \sim C_6$ alkyl, -OH substituted $C_1 \sim C_6$ alkyl.

Further, R_5 and R_6 are independently selected from -OH, -H, methyl, ethyl, propyl, hydroxymethyl, hydroxyethyl, and hydroxypropyl.

Further, R_5 , R_6 are selected from the same group.

Among then, the writing order of the above-defined L1, L2, L3, and L4 linkages corresponds to the proximal nitrogen end to the far nitrogen end from left to right.

The writing order of the above-defined connecting keys of G1, G2, G3, and G4 is from left to right corresponding to the direction of the main chain of formula I from left to right.

The present disclosure provides the compound, or its pharmaceutically acceptable salts, isomers, deuterium substitutes or prodrugs, as nucleic acid delivery carriers.

The present disclosure provides a pharmaceutical composition containing the compound described herein, or its pharmaceutically acceptable salts, isomers, deuterium substitutes or prodrugs, and nucleic acid drugs.

Further, the pharmaceutical composition also contains at least one excipient of neutral phospholipids, steroids, and polyethylene glycol lipids.

In some embodiments, the neutral phospholipids are selected from at least one of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylcholine (DOPC), 1,2-distearoyl-sn-glycero-3-phosphorylethanolamine (DSPE), dimyristoylphosphatidylcholine (DMPC), 1,2-bis(dimethylphosphino)ethane (DMPE), dipalmitoylphosphatidylcholine (DPPC), 1,2-bis(diphenylphosphino)ethane (DPPE), 1,2-dierucoyl-sn-glycero-3-phosphocholine (DEPC), L- α -phosphatidylcholine, hydrogenated (Soy) (HSPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC).

In one embodiment, the neutral phospholipid is DOPE.

In some embodiments, the mole ratio of the compound described herein: neutral phospholipid is 1:1~5:1.

In some embodiments, the steroids are selected from at least one of cholesterol, sitosterol, soybean sterol, wool sterol and ergosterol.

In one embodiment, the steroid is cholesterol.

In some embodiments, the mole ratio of the compound: steroid is 1:2~2:1.

In some embodiments, the pegylated lipids are selected from at least one of DMG-PEG and DSPE-PEG.

Preferably, the pegylated lipid is DMG-PEG2000.

In some embodiments, the mole ratio of the compound: pegylated lipids is 5:1~100:1.

Preferably, the mole ratio of the compound: polyethylene glycol lipid is 10:1~20:1.

In some embodiments, the nucleic acid drug is selected from at least one of DNA, ASO, siRNA, miRNA, mRNA, ribozyme and aptamer.

Further, the nucleic acid drug is mRNA.

Further, the drug composition is prepared into lipid nanoparticles LNP.

The above-mentioned lipid nanoparticles can be used for *in vivo* delivery of nucleic acid drugs such as mRNA to achieve up-regulation or down-regulation of corresponding

genes, or delivery of antigen mRNA to express antigen *in vivo* to achieve immunotherapy, or delivery of mRNA encoding antibody to express antibody *in vivo* and other purposes.

The compounds and derivatives provided herein may be named according to the nomenclature system of IUPAC (International union of pure and applied chemistry) or CAS (Chemical abstracts service, Columbus, OH).

The term "alkyl" is a straight or branched saturated hydrocarbon radical of formula $-C_nH_{(2n+1)}$. C_1 - C_6 alkyl groups include but are not limited to methyl (C_1), ethyl (C_2), n-propyl (C_3), isopropyl (C_3), n-butyl (C_4), tert-butyl (C_4), sec-butyl (C_4), isobutyl (C_4), n-amyl (C_5), 3-amyl (C_5), amyl (C_5), neopentyl (C_5), 3-methyl-2-butyl (C_5), tertiary amyl (C_5), and n-hexyl (C_6). As used herein, a " C_6 - C_{30} (or C_6 - C_{18})alkyl" group means a radical having from 6 to 30(or 6 to 18) carbon atoms in a straight or branched arrangement. In some embodiments, a " C_6 - C_{30} (or C_6 - C_{18})alkyl" group means a radical having from 6 to 30(or 6 to 18) carbon atoms in a straight arrangement. In some embodiments, a " C_6 - C_{30} (or C_6 - C_{18})alkyl" group means a radical having from 6 to 30(or 6 to 18) carbon atoms in a branched arrangement.

The term "alkenyl" is a straight or branched hydrocarbon group containing at least one double bond. Alkenyl groups include but are not limited to vinyl, prop-1-enyl, butyl 1-enyl, butyl 2-enyl, amyl 1-enyl, amyl 2-enyl, amyl 3-enyl, hex-1-enyl, hex-2-enyl, hex-3-enyl, hex-4-enyl. As used herein, a " C_6 - C_{30} (or C_6 - C_{18})alkenyl" group means a radical having from 6 to 30(or 6 to 18) carbon atoms in a straight or branched arrangement. In some embodiments, a " C_6 - C_{30} (or C_6 - C_{18})alkenyl" group means a radical having from 6 to 30(or 6 to 18) carbon atoms in a straight arrangement. In some embodiments, a " C_6 - C_{30} (or C_6 - C_{18})alkenyl" group means a radical having from 6 to 30(or 6 to 18) carbon atoms in a branched arrangement.

The term "alkynyl" is a straight or branched hydrocarbon group containing at least one triple bond. Alkynyl groups include but are not limited to ethynyl, propargyl, butyl 1-alkynyl, butyl 2-alkynyl, amyl 1-alkynyl, amyl 2-alkynyl, amyl 3-alkynyl, hex-1-alkynyl, hex-2-alkynyl, hex-3-alkynyl, hex-4-alkynyl. As used herein, a " C_6 - C_{30} (or C_6 - C_{18})alkynyl" group means a radical having from 6 to 30(or 6 to 18) carbon atoms in a straight or branched arrangement. In some embodiments, a " C_6 - C_{30} (or C_6 - C_{18})alkynyl" group means a radical having from 6 to 30(or 6 to 18) carbon atoms in a straight arrangement. In some embodiments, a " C_6 - C_{30} (or C_6 - C_{18})alkynyl" group means a radical having from 6 to 30(or 6 to 18) carbon atoms in a branched arrangement.

Where suitable substituents are not specifically enumerated, exemplary substituents

include, but are not limited to: C₁₋₅alkyl, C₁₋₅hydroxyalkyl, C₁₋₅haloalkyl, C₁₋₅alkoxy, C₁₋₅ haloalkoxy, halogen, hydroxyl, cyano, amino, -CN, -NO₂, -OR^{c1}, -NR^{a1}R^{b1}, -S(O)_iR^{a1}, -NR^{a1}S(O)_iR^{b1}, -S(O)_iNR^{a1}R^{b1}, -C(=O)OR^{a1}, -OC(=O)OR^{a1}, -C(=S)OR^{a1}, -O(C=S)R^{a1}, -C(=O)NR^{a1}R^{b1}, -NR^{a1}C(=O)R^{b1}, -C(=S)NR^{a1}R^{b1}, -C(=O)R^{a1}, -C(=S)R^{a1}, NR^{a1}C(=S)R^{b1}, -O(C=O)NR^{a1}R^{b1}, -NR^{a1}(C=S)OR^{b1}, -O(C=S)NR^{a1}R^{b1}, -NR^{a1}(C=O)NR^{a1}R^{b1}, -NR^{a1}(C=S)NR^{a1}R^{b1}, phenyl, or 5-6 membered heteroaryl. Each R^{a1} and each R^{b1} are independently selected from -H and C₁₋₅alkyl, optionally substituted with hydroxyl or C₁₋₃alkoxy; R^{c1} is -H, C₁₋₅haloalkyl or C₁₋₅alkyl, wherein the C₁₋₅alkyl is optionally substituted with hydroxyl or C₁₋₃alkoxy.

The term "pharmaceutically acceptable" means that a carrier, excipient, salt, etc., which is usually chemically or physically compatible with the other components that make up a pharmaceutical dosage form and physiologically compatible with the receptor. The term "pharmaceutically acceptable salt" means acid and/or base salts of the compounds described in the patent in association with inorganic and/or organic acids and bases, also including amphoteric ionic salts (inner salts) and quaternary ammonium salts, such as alkyl ammonium salts. These salts can be obtained directly in the final isolation and purification of the compounds. These salts also can be obtained by mixing the above-mentioned compound with acid or base as appropriate (for example, an equivalent amount). These salts may be collected by filtration as precipitation in solution, or by recovery after evaporation of the solvent, or by freeze-drying after reaction in aqueous media. The salts described in the patent may be compounds of hydrochloride, sulfate, citrate, benzoate, hydrobromate, hydrofluorate, phosphate, acetate, propionate, succinate, oxalate, malate, succinate, fumarate, maleate, tartrate or trifluoroacetate.

The present disclosure provides a novel ionizable lipid, whose hydrophilic center is composed of four tertiary amine atoms, and hydrophobic tail is composed of four saturated or unsaturated fat chains. The novel ionizable lipid provided by the present disclosure is positively charged in an acidic environment, and almost un-charged in a neutral and physiological pH environment. Nucleic acid drugs can be transferred by using this property in an acidic buffer system. After the nucleic acid drugs are loaded, the system is adjusted to neutral, so that the lipid nanoparticles are uncharged to avoid adsorption by plasma proteins and achieve higher delivery efficiency and safety.

The scheme of the present disclosure is explained below in combination with embodiments. Those skilled in the field will understand that the following embodiments are

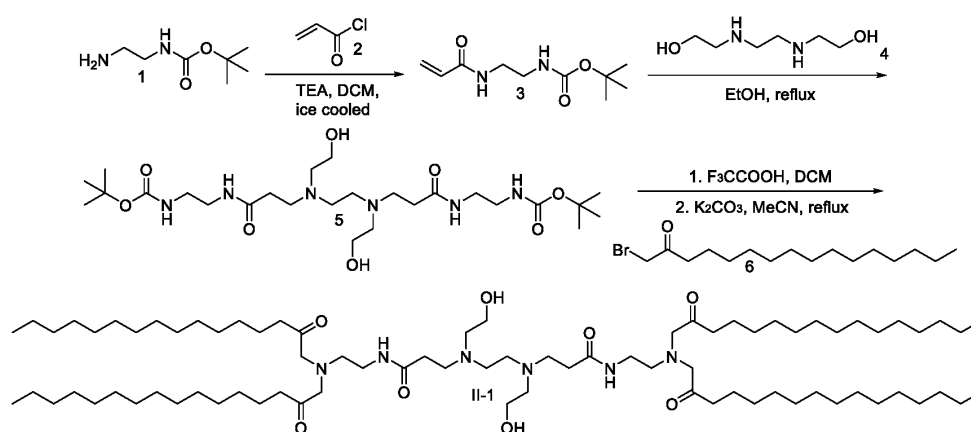
intended only to illustrate the present disclosure and should not be regarded as limiting the scope of the present disclosure. If the specific technology or conditions are not specified in the embodiment, the technology or conditions described in the literature in the field or the product specification shall be followed. Reagents or instruments used without manufacturer are conventional products that can be purchased in the market.

EXAMPLES

Abbreviations

DCM	1,2-Dichloromethane
DIPEA	N-ethyl-N-isopropylpropan-2-amine
EA	Ethyl acetate
eq	equivalent
EtOH	Ethanol
MeOH	Methanol
PE	Petroleum ether
TEA	Triethyl amine
TFA	Trifluoroacetic acid

Example 1 Synthesis of compound II-1



(1) Synthesis of compound 3:

N-Boc-1,2-ethylenediamine (1.0eq) and TEA (2.0eq) were added to a single-necked bottle, dissolved in an appropriate amount of anhydrous DCM, and stirred evenly in an ice-water bath. Separately, acryloyl chloride (1.2eq) was dissolved in an appropriate amount of anhydrous DCM and added to a constant pressure dropping funnel, and the flow rate was

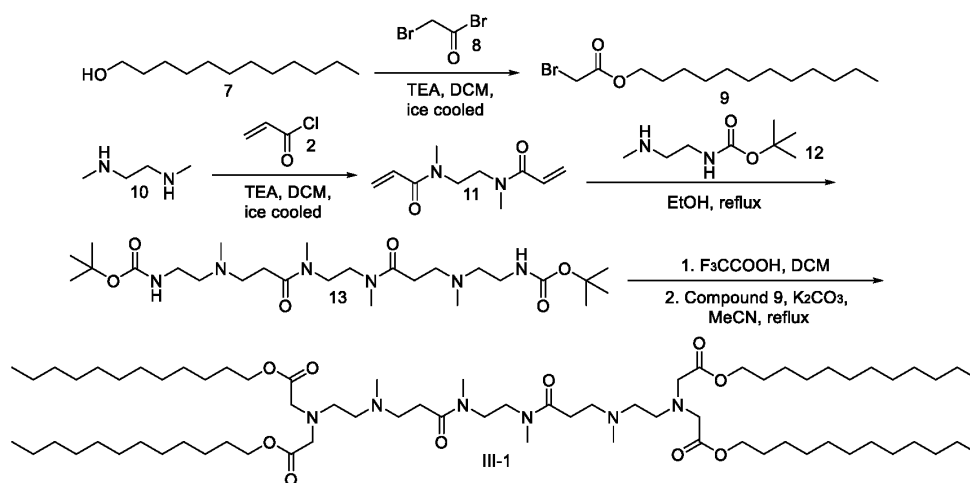
controlled to make it dropwise into the above single-necked bottle and reacted in an ice-water bath for 6h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM: MeOH=50:1), concentrated and dried to obtain a white solid 3 with a yield of 90.5%.

(2) Synthesis of compound 5:

Add N,N'-bis(2-hydroxyethyl)ethylenediamine (1.0eq) and compound 3 (2.0eq) to a single-necked flask and dissolve in an appropriate amount of anhydrous ethanol. Add a condenser tube to the single-necked flask and place it in an oil bath at 80°C. Stir at medium reflux for 12h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=15:1, 0.5% ammonia water), concentrated and dried to obtain pale-yellow solid 5 with a yield of 85.3%.

(3) Synthesis of II-1:

Compound 5 was dissolved in DCM, a sufficient amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spun down to give a yellow oil. The above-mentioned oily substance is dissolved in an appropriate amount of acetonitrile, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline. 1-Bromo-2-hexadecanone (6.0eq) was added to the above reaction solution, a condenser was added to the single-necked flask, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=20:1, 0.5% ammonia water), concentrated and dried to obtain yellow oil II-1 with a yield of 76.8%.

Example 2 Synthesis of compound III-1**(1) Synthesis of compound 9:**

Dodecanol (1.0 eq) and TEA (2.0 eq) were added to a single-necked bottle, dissolved in an appropriate amount of anhydrous DCM, and stirred evenly in an ice-water bath. In addition, bromoacetyl bromide (1.2eq) was dissolved in an appropriate amount of anhydrous DCM and added to a constant pressure dropping funnel, and the flow rate was controlled to make it dropwise into the above single-necked bottle, and reacted in an ice-water bath for 6h. The reaction solvent was spin-dried, the crude product was purified by silica gel column chromatography (PE:DCM=1:1), concentrated and dried to obtain a colorless liquid 9 with a yield of 86.1%.

(2) Synthesis of compound 11:

Add N,N'-dimethylethylenediamine (1.0eq) and TEA (3.0eq) to a single-necked bottle, dissolve in an appropriate amount of anhydrous DCM, and stir evenly in an ice-water bath. Separately, acryloyl chloride (2.5eq) was dissolved in an appropriate amount of anhydrous DCM and added to a constant pressure dropping funnel, and the flow rate was controlled to make it dropwise into the above single-necked bottle, and reacted in an ice-water bath for 6h. The reaction solvent was spin-dried, the crude product was purified by silica gel column chromatography (DCM:MeOH=60:1), and concentrated to dryness to obtain white solid 11 with a yield of 92.5%.

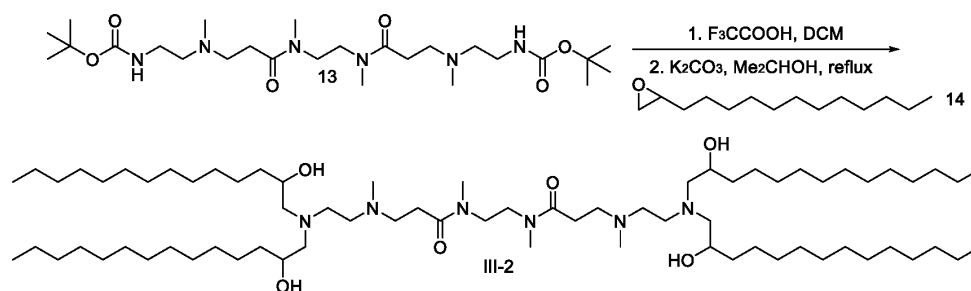
(3) Synthesis of compound 13:

Add compound 11 (1.0eq) and tert-butyl 2-(methylamino)ethylcarbamate (2.0eq) to a single-necked flask and dissolve in an appropriate amount of anhydrous ethanol, add a condenser tube to the single-necked flask and reflux in an oil bath at 80°C Stir for 12h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=20:1, 0.5% ammonia water), concentrated and dried to obtain yellow semi-solid 13 with a yield of 89.1%.

(4) Synthesis of III-1:

Compound 13 was dissolved in DCM, a sufficient amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spun down to give a yellow oil. The above-mentioned oily substance is dissolved in an appropriate amount of acetonitrile, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline.

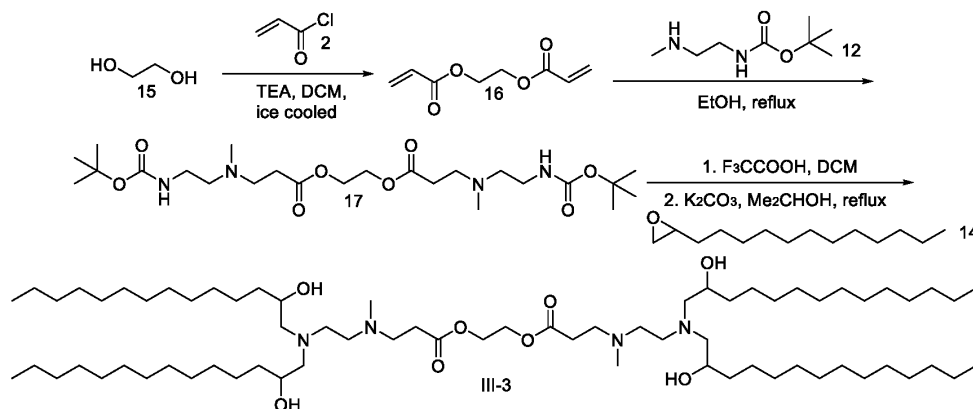
Compound 9 (6.0eq) was added to the above reaction solution, a single-necked flask was added with a condenser tube, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=20:1, 0.5% ammonia water), concentrated and dried to obtain yellow oil III-1 with a yield of 70.8%.

Example 3 Synthesis of compound III-2

Compound 13 was dissolved in DCM, a sufficient amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spun down to give a yellow oil. The above-mentioned oily substance is dissolved in an appropriate amount of isopropanol, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline. 1,2-

Epoxytetradecane (6.0eq) was added to the above reaction solution, a condenser was added to the single-necked flask, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM: MeOH=15:1, 0.5% ammonia water), concentrated and dried to obtain yellow oil III-2 with a yield of 75.1%.

Example 4 Synthesis of compound III-3



(1) Synthesis of compound 16:

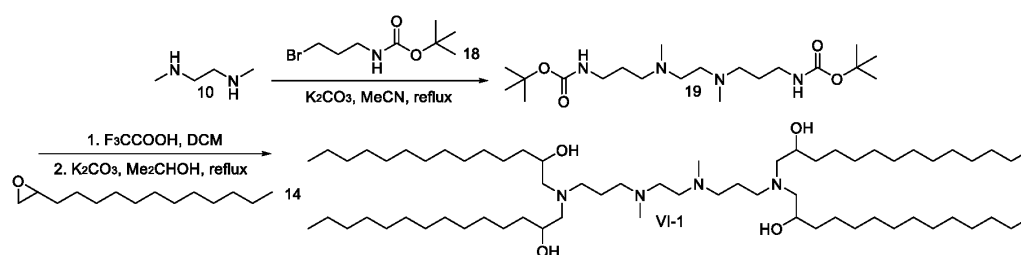
Add ethylene glycol (1.0 eq) and TEA (3.0 eq) to a single-necked bottle, dissolve in an appropriate amount of anhydrous DCM, and stir evenly in an ice-water bath. Separately, acryloyl chloride (2.5eq) was dissolved in an appropriate amount of anhydrous DCM and added to a constant pressure dropping funnel, and the flow rate was controlled to make it dropwise into the above single-necked bottle, and reacted in an ice-water bath for 6h. The reaction solvent was spin-dried, the crude product was purified by silica gel column chromatography (DCM:MeOH=80:1), concentrated and dried to obtain colorless liquid 16 with a yield of 87.5%.

(2) Synthesis of compound 17:

Add compound 16 (1.0eq) and tert-butyl 2-(methylamino)ethylcarbamate (2.0eq) to a single-necked flask and dissolve in an appropriate amount of anhydrous ethanol. Add a condenser tube to the single-necked flask and reflux in an oil bath at 80°C Stir for 12h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=25:1, 0.5% ammonia water), concentrated and dried to obtain light yellow oily product 17 with a yield of 84.4%.

(3) Synthesis of III-3:

Compound 17 was dissolved in DCM, an appropriate amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spun down to give a yellow oil. The above-mentioned oily substance is dissolved in an appropriate amount of isopropanol, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline. 1,2-Epoxytetradecane (6.0eq) was added to the above reaction solution, a condenser was added to the single-necked flask, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=25:1, 0.5% ammonia water), concentrated and dried to obtain a pale-yellow oily product III-3 with a yield of 67.8%.

Example 5 Synthesis of compound VI-1

(1) Synthesis of compound 19:

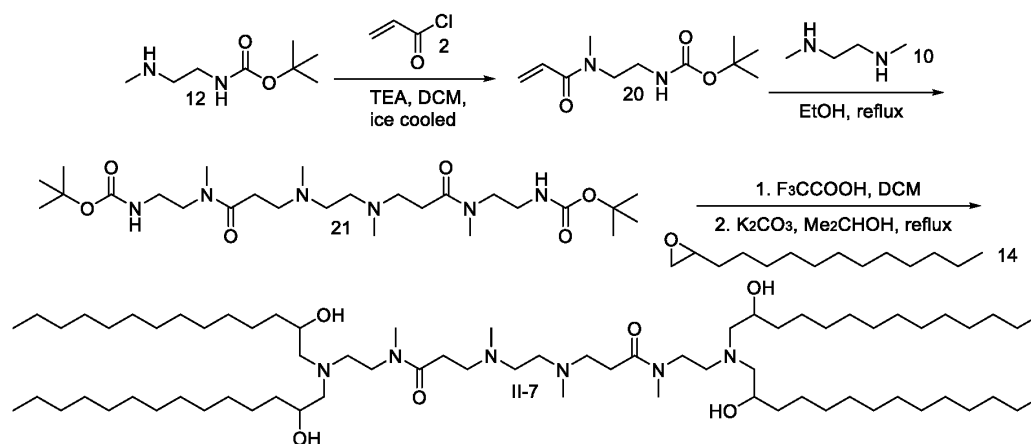
Add N,N'-dimethylethylenediamine (1.0eq) and N-Boc-3-aminopropyl bromide (2.5eq) to a single-necked bottle and dissolve in appropriate amount of acetonitrile. Add a condenser tube to the single-necked bottle and heat it at 90°C. The bath was refluxed and stirred for 12h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=25:1, 0.5% ammonia water), concentrated and dried to obtain light yellow oily product 17 with a yield of 78.8%.

(2) Synthesis of VI-1:

Compound 19 was dissolved in DCM, an appropriate amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spun down to give a yellow oil. The above-mentioned oily substance is dissolved in an appropriate amount of isopropanol, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline.

1,2-Epoxytetradecane (6.0eq) was added to the above reaction solution, a condenser was added to the single-necked flask, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, the crude product was purified by silica gel column chromatography (DCM:MeOH=15:1, 0.5% ammonia water), concentrated and dried to obtain a yellow oil VI-1 with a yield of 70.8%.

Example 6 Synthesis of compound II-7



(1) Synthesis of compound 20:

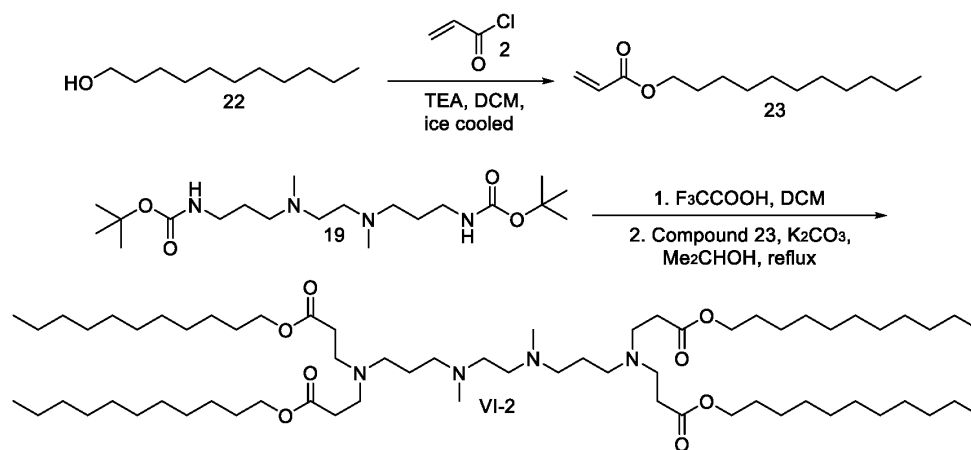
In a single-neck flask, add tert-butyl 2-(methylamino)ethylcarbamate (1.0 eq) and TEA (3.0 eq), dissolve in an appropriate amount of anhydrous DCM, and stir evenly in an ice-water bath. Separately, acryloyl chloride (1.2eq) was dissolved in an appropriate amount of anhydrous DCM and added to a constant pressure dropping funnel, and the flow rate was controlled to make it dropwise into the above single-necked bottle, and reacted in an ice-water bath for 6h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=60:1), concentrated and dried to obtain a white semi-solid 20 with a yield of 89.5%.

(2) Synthesis of compound 21:

Add N,N'-dimethylethylenediamine (1.0eq) and compound 20 (2.0eq) to a single-necked flask and dissolve in an appropriate amount of anhydrous ethanol. Add a condenser tube to the single-necked flask and reflux and stir in an oil bath at 80°C for 12h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=20:1, 0.5% ammonia water), concentrated and dried to obtain yellow oil 21 in a yield of 84.0%.

(3) Synthesis of II-7:

Compound 21 was dissolved in DCM, an appropriate amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spun down to give a yellow oil. The above-mentioned oily substance is dissolved in an appropriate amount of isopropanol, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline. 1,2-Epoxytetradecane (6.0eq) was added to the above reaction solution, a condenser was added to the single-necked flask, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=20:1, 0.5% ammonia water), concentrated and dried to obtain yellow oil II-7 with a yield of 69.1%.

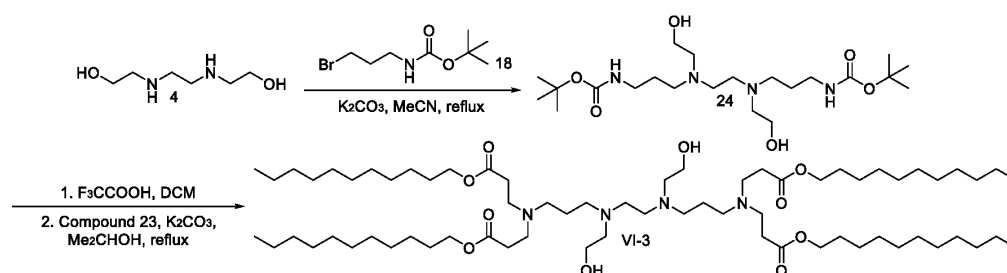
Example 7 Synthesis of compound VI-2

(1) Synthesis of compound 23:

Undecyl alcohol (1.0 eq) and TEA (2.0 eq) were added to a single-necked bottle, dissolved in an appropriate amount of anhydrous DCM, and stirred evenly in an ice-water bath. Separately, acryloyl chloride (1.2eq) was dissolved in an appropriate amount of anhydrous DCM and added to a constant pressure dropping funnel, and the flow rate was controlled to make it dropwise into the above single-necked bottle, and reacted in an ice-water bath for 6h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (PE:EA=2:1), concentrated and dried to obtain a colorless liquid 23 with a yield of 90.0%.

(2) Synthesis of VI-2:

Compound 19 was dissolved in DCM, an appropriate amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spun down to give a yellow oil. The above-mentioned oily substance is dissolved in an appropriate amount of isopropanol, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline. Compound 23 (6.0eq) was added to the above reaction solution, a single-necked flask was added with a condenser tube, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=25:1, 0.5% ammonia water), concentrated and dried to obtain light yellow oil VI-2 with a yield of 74.1%.

Example 8 Synthesis of compound VI-3

(1) Synthesis of compound 24:

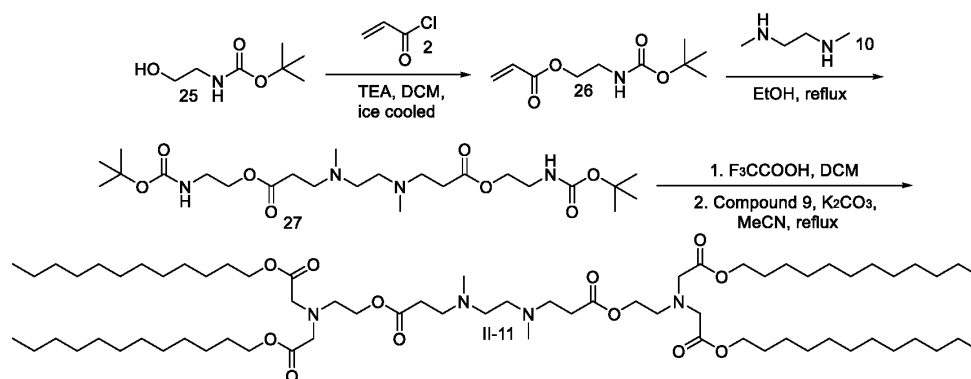
Add N,N'-bis(2-hydroxyethyl)ethylenediamine (1.0eq) and N-Boc-3-aminopropyl bromide (2.5eq) to a single-necked bottle and dissolved in an appropriate amount of acetonitrile, add a condenser to the single-necked bottle, then reflux and stir in an oil bath at 90°C for 12h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=20:1, 0.5% ammonia water), concentrated and dried to obtain pale-yellow oily compound 24 with a yield of 71.2%.

(2) Synthesis of VI-3:

Compound 24 was dissolved in DCM, an appropriate amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spin-dried, obtaining a yellow oily product. The above-mentioned oily substance is dissolved in an appropriate amount of isopropanol, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent

is alkaline. Compound 23 (6.0eq) was added to the above reaction solution, a single-necked flask was added with a condenser, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, the crude product was purified by silica gel column chromatography (DCM:MeOH=15:1, 0.5% ammonia water), concentrated and dried to obtain pale-yellow oil VI-3 with a yield of 69.5%.

Example 9 Synthesis of compound II-11



(1) Synthesis of compound 26:

N-Boc-ethanolamine (1.0eq) and TEA (2.0eq) were added to a single-necked flask, dissolved in an appropriate amount of anhydrous DCM, and stirred evenly in an ice-water bath. Separately, acryloyl chloride (1.2eq) was dissolved in an appropriate amount of anhydrous DCM and added to a constant pressure dropping funnel, and the flow rate was controlled to make it dropwise into the above single-necked bottle and reacted in an ice-water bath for 6h. The reaction solvent was spin-dried, the crude product was purified by silica gel column chromatography (DCM:MeOH=70:1), concentrated and dried to obtain white semi-solid compound 26 with a yield of 85.4%.

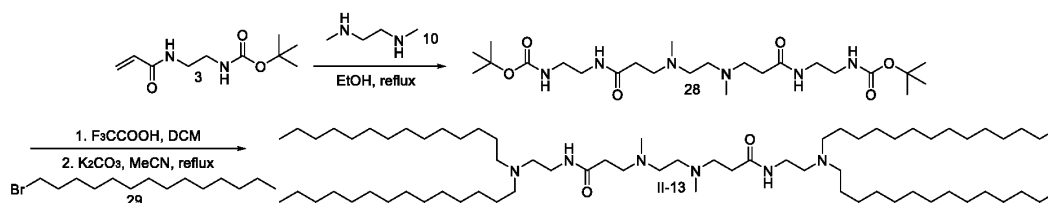
(2) Synthesis of compound 27:

Add N,N'-dimethylethylenediamine (1.0eq) and compound 26 (2.0eq) to a single-necked flask and dissolve in an appropriate amount of anhydrous ethanol. Add a condenser to the single-necked flask, then reflux and stir it in an oil bath at 80°C for 12h. The reaction solvent was spin-dried, the crude product was purified by silica gel column chromatography (DCM: MeOH=25:1, 0.5% ammonia water), concentrated and dried to obtain pale-yellow semi-solid compound 27 with a yield of 82.4%.

(3) Synthesis of II-11:

Compound 27 was dissolved in DCM, a sufficient amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spun down to give a yellow oily product. The above-mentioned oily substance is dissolved in an appropriate amount of acetonitrile, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline. Compound 9 (6.0eq) was added to the above reaction solution, a single-necked flask was added with a condenser, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=30:1, 0.5% ammonia water), concentrated and dried to obtain pale-yellow oily compound II-11 with a yield of 72.1%.

Example 10 Synthesis of compound II-13



(1) Synthesis of compound 28:

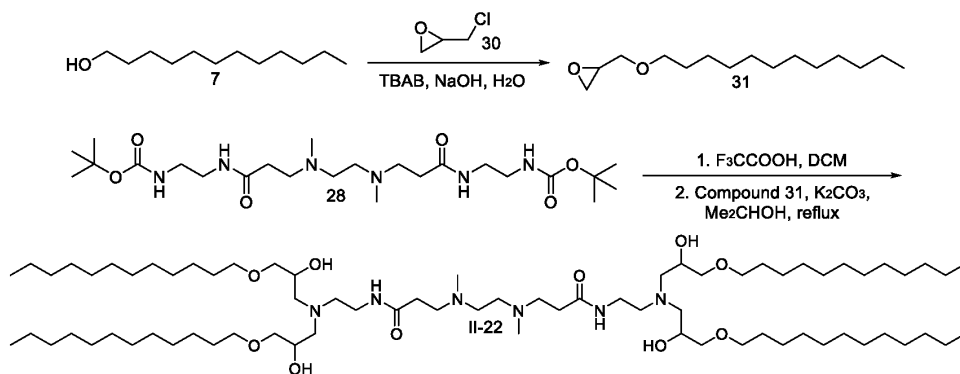
Add N,N'-dimethylethylenediamine (1.0eq) and compound 3 (2.0eq) to a single-necked flask and dissolve in an appropriate amount of anhydrous ethanol. Add a condenser to the single-necked flask, then reflux and stir it in an oil bath at 80°C for 12h. The reaction solvent was spin-dried, the crude product was purified by silica gel column chromatography (DCM:MeOH=20:1, 0.5% ammonia water), concentrated and dried to obtain a pale-yellow solid compound 28 with a yield of 85.9%.

(2) Synthesis of II-13:

Compound 28 was dissolved in DCM, a sufficient amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spin-dried to give a yellow oily product. The above-mentioned oily substance is dissolved in an appropriate amount of acetonitrile, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline. Bromotetradecane (6.0eq) was added to the above reaction solution, a condenser was added to the single-neck flask, and the mixture was refluxed and stirred in an oil bath at

90°C for 36h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM: MeOH=25:1, 0.5% ammonia water), concentrated and dried to obtain pale-yellow semi-solid compound II-13 with a yield of 75.5%.

Example 11 Synthesis of compound II-22



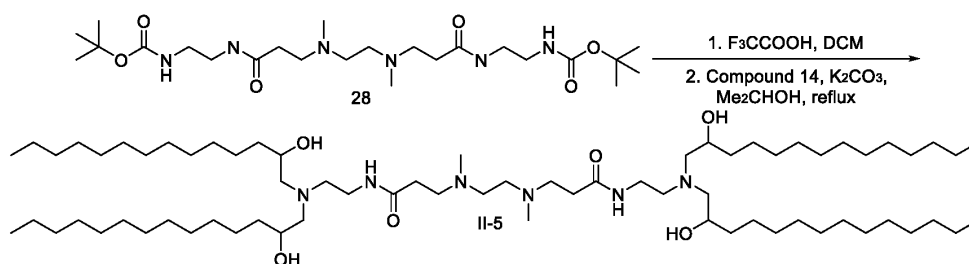
(1) Synthesis of compound 31:

Add dodecanol (1.0eq), epichlorohydrin (2.0eq), sodium hydroxide (2.0eq), tetrabutylammonium bromide, water and cyclohexane to a single-necked flask to form a white emulsion, at room temperature. The reaction was stirred for 4h. After the reaction, suction filtration and wash the filter cake with dichloromethane 2-3 times, the filtrate is dried with anhydrous sodium sulfate, the solvent is spin-dried, and the crude product is purified by silica gel column chromatography (PE/EA=8:1-4:1), a colorless liquid 31 was obtained.

(2) Synthesis of II-22:

Compound 28 was dissolved in DCM, a sufficient amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spin-dried to give a yellow oily product. The above-mentioned oily substance is dissolved in an appropriate amount of isopropanol, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline. Compound 31 (6.0eq) was added to the above reaction solution, a single-neck flask was added with a condenser tube, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=25:1, 0.5% ammonia water), concentrated and dried to obtain a pale-yellow oily compound II-22 with a yield of 69.5%.

Example 12 Synthesis of Compound II-5

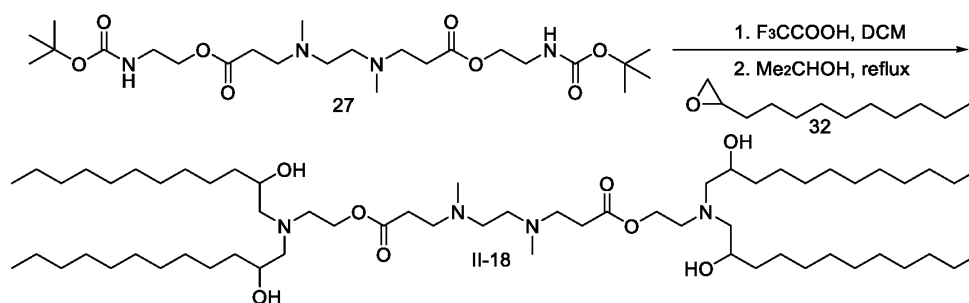


Synthesis of II-5:

Compound 28 was dissolved in DCM, a sufficient amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spun down to give a yellow oil. The above-mentioned oily substance is dissolved in an appropriate amount of isopropanol, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline.

Compound 14 (6.0eq) was added to the above reaction solution, a single-neck flask was added with a condenser tube, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=15:1, 0.5% ammonia water), concentrated and dried to obtain light yellow oil II-5 with a yield of 52.0%.

Example 13 Synthesis of Compound II-18



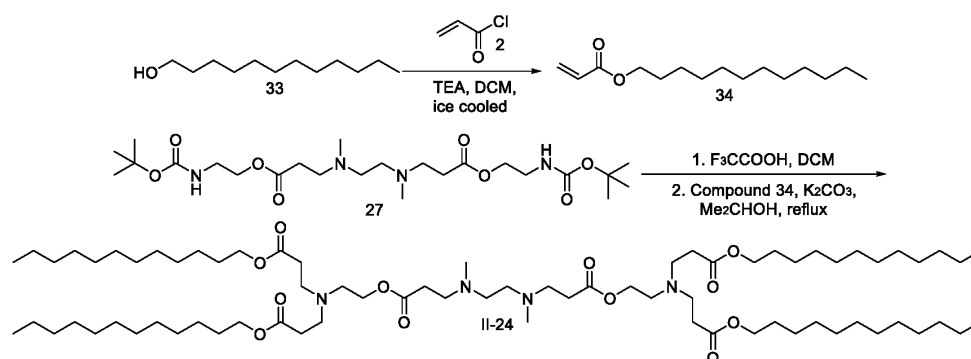
Synthesis of II-18:

Compound 27 was dissolved in DCM, a sufficient amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spun down to give a yellow oil. The above-mentioned oily substance is dissolved in an appropriate amount of isopropanol, a sufficient amount of anhydrous potassium carbonate is added under stirring,

and the mixture is stirred at room temperature until the reaction solvent is alkaline.

Compound 32 (6.0eq) was added to the above reaction solution, a condenser was added to the single-neck flask, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=15:1, 0.5% ammonia water), concentrated and dried to obtain light yellow oil II-18 with a yield of 61.7%.

Example 14 Synthesis of Compound II-24



(1) Synthesis of Compound 34:

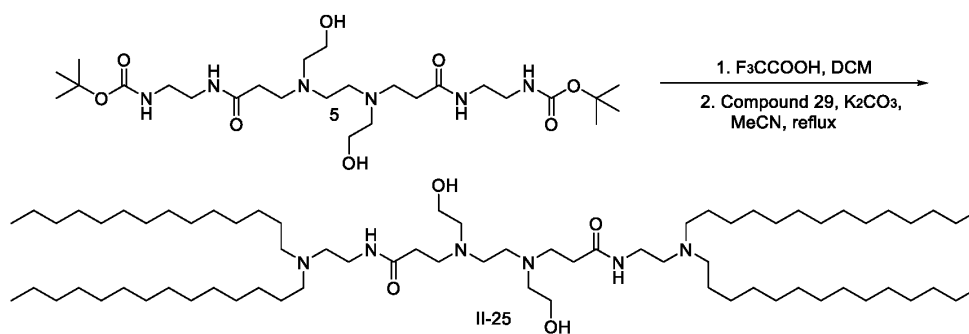
Dodecanol (1.0 eq) and TEA (2.0 eq) were added to a single-necked bottle, dissolved in an appropriate amount of anhydrous DCM, and stirred evenly in an ice-water bath. Separately, acryloyl chloride (1.2eq) was dissolved in an appropriate amount of anhydrous DCM and added to a constant pressure dropping funnel, and the flow rate was controlled to make it dropwise into the above single-necked bottle and reacted in an ice-water bath for 6h. The reaction solvent was spin-dried, the crude product was purified by silica gel column chromatography (PE:EA=2:1), concentrated and dried to obtain a colorless liquid 34 with a yield of 91.0%.

(2) Synthesis of II-24:

Compound 27 was dissolved in DCM, an appropriate amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spun down to give a yellow oil. The above-mentioned oily substance is dissolved in an appropriate amount of isopropanol, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline. Compound 34 (6.0eq) was added to the above reaction solution, a single-necked flask was

added with a condenser tube, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=25:1, 0.5% ammonia water), concentrated and dried to obtain pale-yellow oil II-24 with a yield of 70.2%.

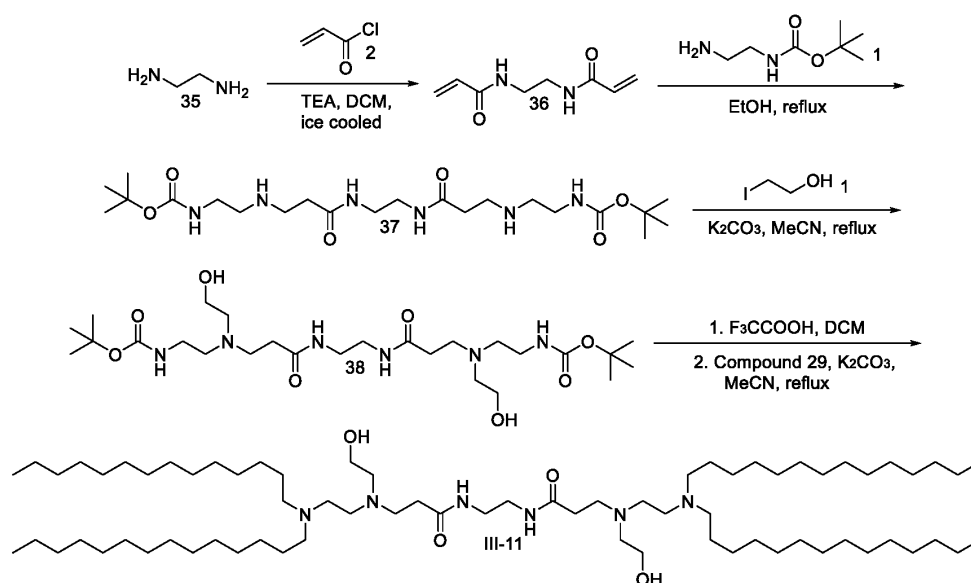
Example 15 Synthesis of Compound II-25



Synthesis of II-25:

Compound 5 was dissolved in DCM, an appropriate amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spun down to give a yellow oil. The above-mentioned oily substance is dissolved in an appropriate amount of acetonitrile, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline.

Compound 29 (6.0eq) was added to the above reaction solution, a condenser was added to the single-necked flask, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, the crude product was purified by silica gel column chromatography (DCM:MeOH=20:1, 0.5% ammonia water), concentrated and dried to obtain light yellow oil II-25 with a yield of 65.3%.

Example 16 Synthesis of Compound III-11**(1) Synthesis of Compound 36:**

Add ethylenediamine (1.0 eq) and TEA (3.0 eq) to a single-necked bottle, dissolve in an appropriate amount of anhydrous DCM, and stir evenly in an ice-water bath. Separately, acryloyl chloride (2.5eq) was dissolved in an appropriate amount of anhydrous DCM and added to a constant pressure dropping funnel, and the flow rate was controlled to make it dropwise into the above single-necked bottle, and reacted in an ice-water bath for 6h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=80:1), concentrated and dried to obtain a white solid 36 with a yield of 89.6%.

(2) Synthesis of Compound 37:

Compound 36 (1.0eq) and Boc-ethylenediamine (2.5eq) were added to the single-necked flask and dissolved in an appropriate amount of absolute ethanol. A condenser was added to the single-necked flask, and the mixture was refluxed and stirred in an oil bath at 80°C for 12h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=15:1, 0.5% ammonia water), concentrated and dried to obtain light yellow oily product 17 with a yield of 80.4%.

(3) Synthesis of Compound 38:

Compound 37 (1.0eq) and iodoethanol (2.5eq) were added to a single-neck flask, dissolved in an appropriate amount of anhydrous acetonitrile, an appropriate amount of potassium carbonate was added, and the mixture was stirred at room temperature overnight. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=10:1, 1% ammonia water), concentrated and dried to obtain pale-yellow oil 38 in a yield of 65.4%.

(4) Synthesis of III-11

Compound 38 was dissolved in DCM, an appropriate amount of TFA was added under stirring, and the reaction was carried out at room temperature for 6 h. TFA/DCM was spun down to give a yellow oil. The above-mentioned oily substance is dissolved in an appropriate amount of acetonitrile, a sufficient amount of anhydrous potassium carbonate is added under stirring, and the mixture is stirred at room temperature until the reaction solvent is alkaline. Compound 29 (6.0eq) was added to the above reaction solution, a condenser was added to the single-necked flask, and the mixture was refluxed and stirred in an oil bath at 90°C for 36h. The reaction solvent was spin-dried, and the crude product was purified by silica gel column chromatography (DCM:MeOH=15:1, 1% ammonia water), concentrated and dried to obtain a pale-yellow oily product III-11 with a yield of 57.2%.

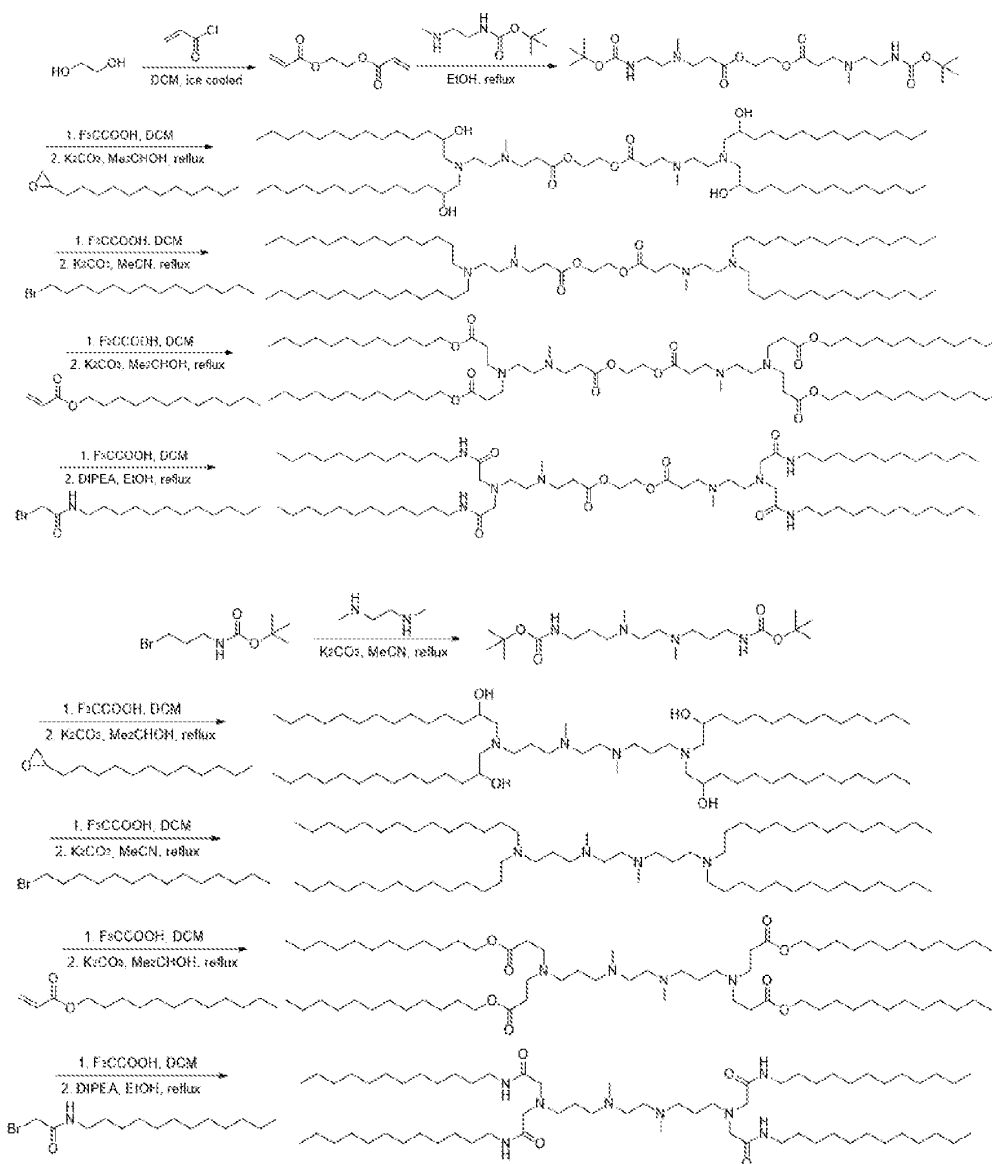
Example 17 Syntheses of Other Exemplified Compounds

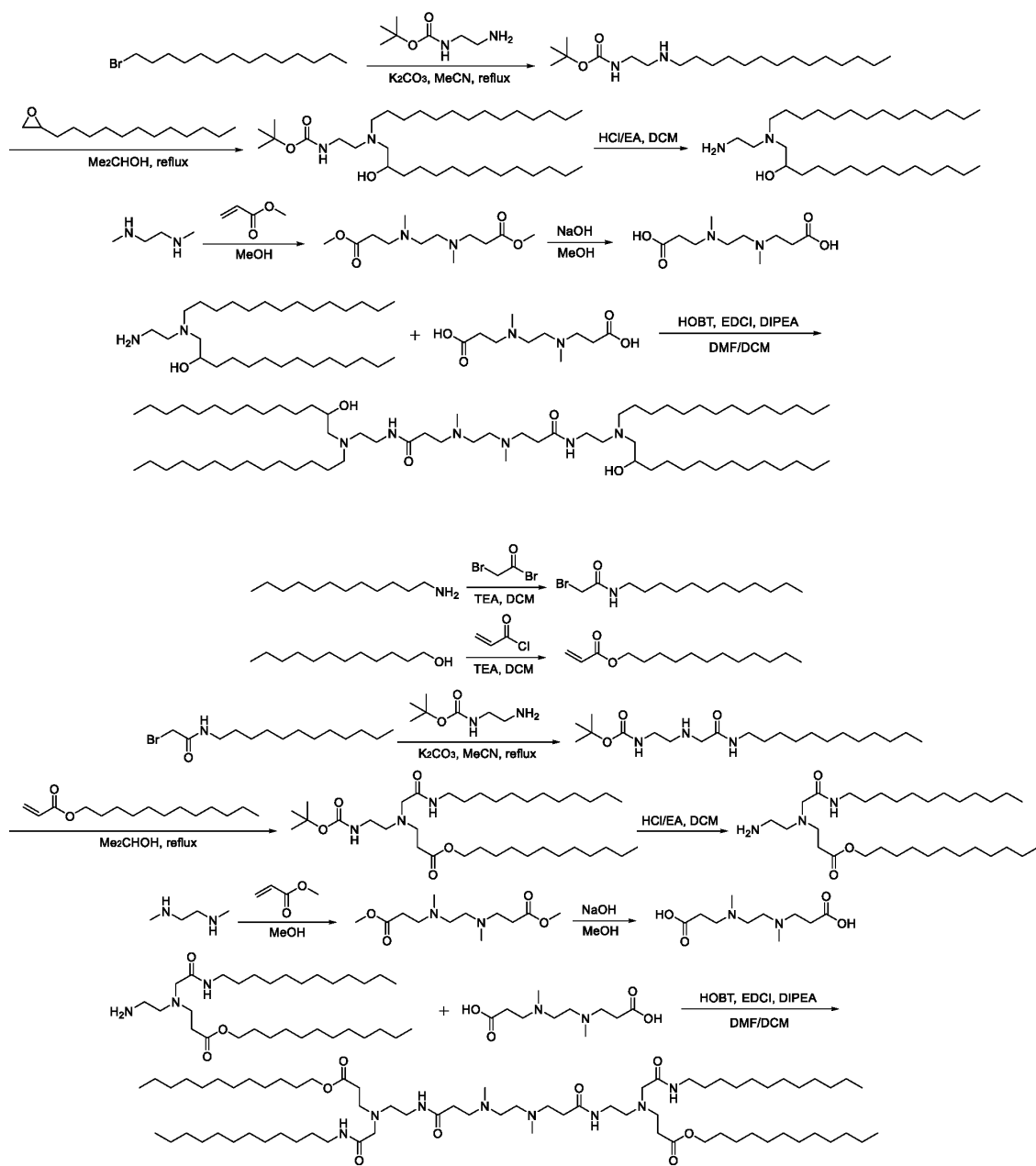
The other exemplified compounds disclosed herein were prepared by the similar procedures described in Examples 1-16 with different starting materials.















Example 18 Preparation of a compound II-1 lipid nanoparticles solution by thin-film hydration

Film formation: The lipid compound II-1 obtained in Example 1, DOPE, cholesterol, and DMG-PEG2000 were dissolved with absolute ethanol to form stock solutions with concentrations of 20 mg/mL, 10 mg/mL, 20 mg/mL, and 10 mg/mL, respectively. The stock solutions were refrigerated for later use. With the above-mentioned stock solutions, the four reagents were mixed to form about 3 mL of a solution by the ratio of 40:10:47.5:2.5 (mol/mol), in which the concentration of lipid compound II-1 was about 5 mg/mL. The solution was transferred into a suitable sized eggplant-shaped bottle. Finally, the solvent was removed by rotary evaporation at 37°C on an evaporator to form a film.

Hydration: a 3 mL of 10 mM citrate buffer solution was added to the above-mentioned eggplant-shaped flask with the film formed, and the film was hydrated by rotating at 60°C on a rotary evaporator. The above-mentioned hydration solution was transferred to a suitable container and sonicated with a probe sonicator to make it uniform. A uniform and clear lipid nanoparticle (LNP) solution was obtained, wherein the concentration of lipid compound II-1 was 3 mg/mL.

Example 19 Preparation of II-1 LNP@mRNA

The basic structural units of nucleic acid molecules such as DNA, siRNA, and mRNA are deoxynucleotides or ribonucleotides. The phosphate groups in the nucleotides dissociate into phosphate ions, which make the nucleic acid molecules negatively charged. In Example 18, in the citrate buffer system of the LNP solution, the ionizable lipid II-1 in the lipid nanoparticles was ionized into cations in an acidic environment, so that the nanoparticles were positively charged to absorb negatively charged nucleic acid drugs.

II-1 LNP and Luciferase mRNA were used as examples to prepare LNP@mRNA. The specific method is as follows: II-1 LNP obtained in Example 18 (the concentration of II-1 is 3 mg/mL) was used in an incubation method to prepare II-1 LNP@mRNA. To prepare LNP@mRNA with a mass ratio of ionizable lipid to mRNA of 10:1, 33 µL of II-1 LNP was labelled as phase A (the mass of II-1 is 100 µg); 10 µg of Luc mRNA was added to RNase Free water and mixed well to obtain phase B (total volume is 67 µL); B was added to A, mixed by pipetting up and down with the tip of the gun, and incubated for 10 min at room temperature to obtain a LNP@mRNA solution with a mass ratio of ionizable lipid to mRNA of

10:1, in which the concentration of mRNA is 0.1 mg/mL. Similarly, to prepare II-1 LNP@mRNA with a mass ratio of 15:1, the volume of phase A was 50 μ L (the mass of II-1 was 100 μ g), and the volume of phase B was 50 μ L. The same method was used for other LNP@mRNAs with different mass ratios.

The above experimental method can be scaled up in the same proportion to prepare a larger volume of LNP@mRNA solution.

Example 20 Investigation II-1 LNP nucleic acid loading capacity

In Example 19, II-1 LNP@mRNA was prepared. Further, it was necessary to investigate the loading capacity of different ionizable lipid nanoparticles disclosed herein for nucleic acid molecules, to investigate the ratio of ionizable lipids to nucleic acid molecules. II-1 LNP and mRNA were used as examples, the loading capacity of ionizable lipid nanoparticles for nucleic acid molecules was investigated.

(1) Preparation of denaturing agarose gel

36 mL of RNase-free water and 0.4 g of agarose were placed in a conical flask, heated in a microwave oven for 2 min, cooled to about 60 °C, added with 4 mL of 10×MOPS (4-morpholinepropanesulfonic acid) and mixed, and then added with 7.5 mL of 37% formaldehyde and mixed evenly. The mixture was poured into a gel tank, the thickness of the gel was controlled to be about 0.5 cm. A comb was inserted into the gel tank and was taken out after solidification. The gel was placed into an electrophoresis tank, and the newly prepared 1×MOPS electrophoresis buffer was added to the electrophoresis tank to cover the gel.

(2) Preparation of electrophoresis sample

To 0.5 μ L mRNA (0.5 μ g) mixed with 4.5 μ L RNase-free water, or 5.0 μ L (0.5 μ g mRNA) of II-1 LNP@mRNA prepared in Example 19 with different mass ratios, was added 5 μ L of formaldehyde loading buffer with ethidium bromide, heated at 70 °C for 5 min, and then centrifuged at 4 °C for a short period of time.

(3) Gel electrophoresis

The samples were added to the gel wells with a loading volume of 10 μ L and the electrophoresis conditions were set to 200 V (current at 300 mA, power at 60 W). When the indicator front reached 2/3 of the gel (about 25 minutes), electrophoresis was stopped, the gel was taken out, and placed in a gel imager for observation. The results are shown in Figure

1.

As shown in Figure 1, in the II-1 LNP@mRNA solution, with a mass ratio of ionizable lipid II-1 to Luc mRNA being 10:1, the complete encapsulation of mRNA was achieved.

Example 21 Formulation characterization of II-1 LNP@mRNA

An appropriate amount of the LNP solution prepared in Example 19 was diluted 100 times with purified water, and the particle size (Size), particle size distribution (PDI) and point (Zeta potential) of the LNP solution were measured in a laser particle size analyzer. The results showed that the particle size of II-1 LNP@mRNA was 102.3 nm, the PDI was 0.195, the potential was 31.2 mV, and the preparation properties were stable.

The particle size and point (Zeta potential) test results are shown in Figure 2.

Example 22 Morphological characterization of II-1 LNP@mRNA

Sample preparation: the LNP solution prepared in Example 19 was diluted with purified water to a total lipid material concentration of about 1 mg/mL. The diluted LNP solution was dropped onto a special copper mesh, let it stand for 3 minutes, and the excess LNP solution was removed with filter paper. The diluted LNP solution was negatively stained by adding a 2% phosphotungstic acid dye solution dropwise for 5 min, and then the excess dye solution was removed with filter paper and left to dry.

Photograph: After air-drying, the morphology of II-1 LNPs was observed under a transmission electron microscope and photographed.

II-5 LNP@mRNA, III-5 LNP@mRNA, VI-1 LNP@mRNA were prepared according to the methods described in Examples 18 and 19, and the mass ratios of ionizable lipid to mRNA were all 10:1. The samples were prepared and photographed according to the above method. The microscopic morphology of II-1 LNP@mRNA, II-5 LNP@mRNA, III-5 LNP@mRNA and VI-1 LNP@mRNA is shown in Figure 3.

Example 23 One-step microfluidic preparation of mRNA-loaded ionizable lipid nanoparticles solution

II-7 LNP@mRNA was used as an example. It was prepared by microfluidic technology. The formulation was composed of II-7, DOPE, cholesterol, DMG-PEG2000 and mRNA, and the mass ratio of II-7 and mRNA was set to 10:1 to prepare an organic phase and an aqueous phase.

II-7, DOPE, cholesterol and DMG-PEG2000 were dissolved in absolute ethanol to prepare a certain volume of an organic phase with a molar ratio of 40:10:47.5:2.5. In the meantime, Luc mRNA was diluted with RNase-Free water to obtain a certain volume of an aqueous phase. The volume ratio of the aqueous phase to the organic phase was 3:1. II-7 LNP loaded with mRNA was prepared by a one-step microfluidic device. The instrument parameters were set as follows: ratio of aqueous phase to organic phase was fixed at 3:1 and the flow rate was fixed at 9 mL/min. The initial microfluidic preparation was ultrafiltered with phosphate buffered saline (PBS) to remove ethanol, and the mRNA concentration of the final preparation was controlled to be 0.1 mg/mL, to obtain II-7 LNP@mRNA.

Example 24 Quant-iT™ RiboGreen™ Kit for detection of encapsulation efficiency

The encapsulation of mRNA by the LNP preparations obtained in Examples 22 and 23 was measured by a Quant-iT™ RiboGreen™ RNA detection kit using a method described in Heyes *et al.*, Journal of Controlled Release, 107:276-287 (2005). It was found that encapsulation rates of mRNA in II-1 LNP@mRNA, II-5 LNP@mRNA, III-5 LNP@mRNA, VI-1 LNP@mRNA and II-7 LNP@mRNA preparations were 86.1%, 82.8%, 85.0%, 78.6% and 90.5%, respectively. These results demonstrate that the ionizable lipids disclosed herein, which were obtained by various preparation methods, have good encapsulation efficiency for mRNA.

Example 25 Preparation of MC3 LNP@mRNA

DLin-MC3-DMA (MC3) is a cationic lipid used in the marketed siRNA drug Patisiran (Onpattro) and is often used as a positive control for nucleic acid loading materials. According to the method of example 23, MC3 LNP@mRNA was prepared with MC3 as the load material of nucleic acid, wherein includes: MC3: DSPC: Cholesterol: DMG-PEG2000 = 50: 10: 37.5: 2.5; the mass ratio of MC3 to mRNA was 10:1, and the concentration of preparation mRNA was 0.1 mg/mL. MC3 LNP@mRNA was used as a positive control for ionizable lipid nanoparticles loaded with nucleic acids of the present disclosure.

Example 26 LNP@GFP mRNA transfect into DC2.4 cells

The foregoing examples demonstrate the pharmaceutical properties of ionizable lipid nanoparticles of the present disclosure. The *in vitro* effects of LNP@mRNA of the present disclosure were demonstrated as follows.

II-13 LNP@GFP mRNA, III-9 LNP@GFP mRNA and IV-4 LNP@GFP mRNA were prepared according to the method described in Example 23, in which the mass ratios of ionizable lipids to GFP mRNA were all 10:1, and the mRNA concentrations in the preparation were all 0.1mg/mL. MC3 LNP@GFP mRNA was prepared according to Example 25.

DC2.4 cells were collected in logarithmic growth phase, and following medium suspending, the cell density was adjusted to 20×10^4 cells /mL. 0.5 mL complete culture medium and 0.5 mL cell suspensions were added to each well and were mixed in the 6-well plate to make the cell density 10×10^4 /mL/ well.

After 18-24 h incubation, the medium in the 6-well plate was changed with 1mL liquid complete culture medium. MC3 LNP-GFP mRNA, II-13 LNP@GFP mRNA, III-9 LNP@GFP mRNA and IV-4 LNP@GFP mRNA, which loaded 1 μ g GFP mRNA, were added into each well of the six-well plate with 3 wells repetitions.

The transfection effects of different preparations were observed and detected by inverted fluorescence microscope and flow cytometry after 24h of administration. The results were shown in Figure 4.

The average GFP positive rate of DC2.4 cells transfected by MC3 LNP@GFP mRNA, II-13 LNP@GFP mRNA, III-9 LNP@GFP mRNA, IV-4 LNP@GFP mRNA, were 45.09%, 82.61%, 61.75% and 90.16%, respectively.

The results showed that the ionizable lipids and nanoparticles of the present disclosure have stronger transfection ability *in vitro* than MC3.

Example 27 The expression and distribution of LNP@Luc mRNA *in vivo*

The effects of ionizable lipid compounds of the present disclosure and the corresponding nanoparticles to deliver mRNA were further verified *in vivo*.

II-11 LNP@Luc mRNA, III-6 LNP@Luc mRNA and V-2 LNP@Luc mRNA were prepared according to the method described in Example 23, in which the mass ratio of ionizable lipids to Luc mRNA was 10:1, and the mRNA concentration in the preparation was 0.1mg/mL. MC3 LNP@Luc mRNA was prepared according to Example 25.

The mRNA expression and distribution were studied in Balb/C male mice.

The above-mentioned four preparations were injected intravenously through the mouse tail vein with a dose of $10\mu\text{g}/100\mu\text{L}$ mouse (normal saline group was injected with $100\mu\text{L}$ normal saline), 3 mice/group. After the injection, the mice were free to eat and drink. After 6 hours of administration, $200\mu\text{L}$ luciferin substrate was injected to mice intraperitoneally. The mice were euthanized after 15 min of luciferin substrate injection, the liver, the spleen and the lung were separated, and the expression and *in vivo* distribution of Luc mRNA were observed using a small animal imaging system, as shown in Figure 5. The results show that the ionizable lipid nanoparticles of the present disclosure have stronger total fluorescence, *i.e.*, stronger effects of *in vivo* mRNA expression than MC3.

Example 28 Immune antitumor effect of LNP@OVA mRNA vaccine.

Based on the applications of Examples 26 and 27, the ionizable lipid compounds and their nanoparticles of the present disclosure can be used as mRNA vaccine delivery systems. Further, the ionizable lipid nanoparticle loaded OVA mRNA of the present disclosure was tested in an immune anti-tumor therapy with E.G7 model mice.

II-9 LNP@OVA mRNA, III-8 LNP@OVA mRNA, II-22 LNP@OVA mRNA and VI-4 LNP@OVA mRNA were prepared according to the method described in Example 23, in which the mass ratio of ionizable lipids to OVA mRNA was 10:1. The mRNA concentration in the preparation was 0.1mg/mL . MC3 LNP@OVA mRNA was prepared according to Example 25.

Newly purchased female C57BL/6 mice were labeled and randomly assigned. Each group had 10 mice, and groups including normal saline group, MC3 LNP@OVA mRNA group, II-9 LNP@OVA mRNA group, III-8 LNP@OVA mRNA group, II-22 LNP@OVA mRNA group, VI-4 LNP@OVA mRNA group. The mice were injected with tumors after one week of adaptation. The induction process was as following description: the cells were collected at the logarithmic growth stage and were washed with sterile PBS. The supernatant was discarded after centrifugation, and the cell density was adjusted to $10\times 10^6/\text{mL}$ by adding sterile PBS. Each 6-week-old female C57BL/6 mouse was subcutaneously injected with $100\mu\text{L}$ E.G7 cells at the right rib. The growth status and size of subcutaneous tumors were observed. Compared with normal mice, there were no significant differences of spirit, activity, appetite, urine or feces reaction in tumor-bearing mice.

The Day of tumor grafting was marked as Day 0, and the appearance of tumor mass indicates

successful modeling. At Day 8, mRNA vaccine was injected into caudal vein (10 μ g/100 μ L/time/mouse, normal saline group was injected with 100 μ L normal saline), and mice were vaccinated every week for 3 times. From Day 8, tumor volume was measured every 2 days. The maximum diameter (a) of the tumor was firstly measured, and then the longest diameter perpendicular to the maximum diameter line (b) was measured, in mm. Tumor volume was calculated according to the following formula $V(\text{mm}^3)=ab^2/2$ and tumor volume growth curve of each mouse and average tumor volume growth curve of each group were recorded. On Day 24, the mice were euthanized, the tumor and organs were weighed after separation and were recorded. The result was shown in Figure 6.

The results show that the ionizable lipids and nanoparticles of the present disclosure have stronger immune anti-tumor effects than MC3, and are promising in the field of mRNA vaccine delivery.

Example 29 Safety evaluation of intramuscularly injected LNP@OVA mRNA vaccines

Furthermore, the injection site reaction of ionizable lipids and their nanoparticles in intramuscular administration was investigated.

III-3 LNP@OVA mRNA and VI-2 LNP@OVA mRNA were prepared according to the method described in Example 23, in which the mass ratio of ionizable lipids to OVA mRNA was 10:1, and the mRNA concentration in the preparation was 0.5mg/mL. MC3 LNP@OVA mRNA was prepared, according to Example 25.

Newly purchased SD rats were randomly divided to 4 groups with 3 rats in each group, including normal saline group, III-3 LNP@OVA mRNA group, VI-2 LNP@OVA mRNA group and MC3 LNP@OVA mRNA group, respectively. After one week of adaptive culture, the lateral hairs of the hind legs of the rats were removed, and 500 μ L mRNA preparation (0.5mg/mL) was intramuscularly injected into each side of the rats, *i.e.*, the dose was 0.5mg/mouse. In the saline group, 500 μ L normal saline was intramuscularly injected into both sides, which was recorded as D0 on the day of administration. Mice were secondly injected in D8 with the same dose as D0. The changes of injection site were observed and recorded every other day after the first injection. The result is shown in Figure 7.

The results showed that the injection site inflammation of MC3 group was severe, and III-3 and VI-2 groups had similar results with the normal saline group with basically no inflammation. The safety of ionizable lipids and nanoparticles of the present disclosure is

higher than MC3 and has good clinical transformation prospect.

Example 30 Preparation of LNP loaded with Luc mRNA by microfluidics

II-5 LNP@Luc mRNA was used as an example. LNP@mRNA was prepared by microfluidic technology. The formulation was composed of II-5, DSPC, Cholesterol, DMG-PEG2000 and mRNA, and the mass ratio of II-5 and mRNA was set to 10:1 to prepare the organic phase and the aqueous phase. Dissolve II-5, DSPC, Cholesterol and DMG-PEG2000 in absolute ethanol and prepare a certain volume of organic phase in a molar ratio of 50:10:38.5:1.5, and simultaneously prepare Luc mRNA with RNase-Free water. A certain volume of aqueous phase, in which the volume ratio of aqueous phase to organic phase is 3:1, was used to prepare II-5LNP preparation loaded with mRNA in one step by a microfluidic nano-preparation apparatus. The instrument parameters are set as follows: volumeration of aqueous phase to organic phase was fixed at 3:1 and the flow rate was fixed at 9 mL/min. The initial microfluidic preparation was ultrafiltered with phosphate buffered saline (PBS) to remove ethanol, and the mRNA concentration of the final preparation was controlled to be 0.1 mg/mL to obtain II-5 LNP@mRNA.

Example 31 Luciferase expression of LNP@Luc mRNA via intramuscular injection

Further, the ability of the ionizable lipid compounds and nanoparticles of the present disclosure to deliver mRNA was verified *in vivo*.

II-5 LNP@Luc mRNA, II-18 LNP@Luc mRNA, II-24 LNP@Luc mRNA, II-25 LNP@Luc mRNA, III-3 LNP@Luc mRNA, III-11 LNP@Luc mRNA were prepared according to the method described in Example 30, wherein the mass ratio of ionizable lipid to Luc mRNA was 15:1, and the concentration of mRNA in the preparation was 0.1 mg/mL. According to Example 25, MC3 LNP@Luc mRNA was prepared. BALB/C male mice were used to conduct mRNA expression and distribution experiments. The above four preparations were intramuscularly injected, 20 μ g/100 μ L/mice (100 μ L normal saline was injected in the normal saline group), 3 rats/group, and the mice were free to eat and drink after injection. 8h after administration, intraperitoneal injection of luciferin substrate 3mg. After 15 min of Luciferin substrate injection, the *in vivo* expression of Luc mRNA was observed using a small animal *in vivo* imaging system, as shown in Figure 8. The results show that the ionizable lipid nanoparticles of the present disclosure have stronger total fluorescence than MC3, that is, the ability to express mRNA *in vivo* is stronger than that of MC3.

Example 32 Encapsulation efficiency detected by Quant-iT™ RiboGreen™ kit

Quant-iT™ RiboGreen™ RNA detection kit was used to determine the encapsulation efficiency of mRNA by LNP preparations in Example 31. The results showed that II-5 LNP@Luc mRNA, II-18 LNP@Luc mRNA, II-24 LNP@Luc mRNA, II-25 LNP@Luc mRNA, III-3 LNP@Luc mRNA, III-11 LNP@Luc mRNA. The encapsulation rates of mRNA in the preparations were 88.5%, 89.4%, 85.7%, 90.5%, 88.0% and 87.9%, respectively. It shows that the LNP prepared by the ionizable lipid provided in the present disclosure has a good encapsulation efficiency for mRNA.

Example 33 Preparation of LNP@S mRNA by microfluidic method

Further, the ionizable lipid of the present disclosure is applied to the novel coronavirus mRNA vaccine, and the mRNA encoding the S protein is designed for SARS-CoV-2. II-5 LNP@S mRNA, II-18 LNP@S mRNA, II-24 LNP@S mRNA, II-25 LNP@S mRNA, III-3 LNP@S mRNA, III-11 LNP@S mRNA were prepared according to the method described in Example 30, wherein the mass ratio of ionizable lipid to Luc mRNA was 15:1, and the concentration of mRNA in the preparation was 0.1 mg/mL.

Example 34 Immunization protocol of SARS-CoV-2 mRNA vaccines

(1) Grouping and administration of mice: BALB/c male mice were randomly divided into groups according to the preparation. In each immunization, 100 μ L of mice were injected into the tail vein, that is, 10 μ g of S mRNA per mouse. PBS was used as negative Control. Formulations are grouped as: II-5 LNP@S mRNA, II-18 LNP@S mRNA, II-24 LNP@S mRNA, II-25 LNP@S mRNA, III-3 LNP@S mRNA, and III-11 LNP@S mRNA.

(2) Second immunization and sample collection: The first immunization was recorded as Day 0, and blood was collected after two weeks before the second immunization, and blood was collected every two weeks thereafter.

Example 35 Detection of specific antibody titers by enzyme-linked immunosorbent assay

(1) Antigen coating: RBD protein (Delta, WT or Omicron) was prepared into antigen protein solution with 1 $\times$ coating working solution, and 100 μ L was added to each well. To avoid the existence of air bubbles, the mixture was placed in the refrigerator at 4 $^{\circ}$ C overnight.

(2) BSA blocking: After the antigen was coated overnight, discard the liquid in the coated

ELISA plate, add 300 μ L of 1 $\times$ washing working solution to each well, discard the liquid in the ELISA plate, and repeat the plate washing 4 times. BSA was made up as blocking solution with 1X wash solution. After washing the plate, add 100 μ L of blocking solution to each well and incubate at 25°C for 4 h.

(3) Serum dilution and sample loading: centrifuge the orbital blood of the immunized mice, aspirate the serum, and inactivate at 60°C for 30 min. Using the antibody diluent, take 100-fold as the initial dilution, and carry out 2-fold serial dilution. After the closure, the plate was washed 4 times. Add 100 μ L of serum sample diluent to each well of the washed ELISA plate. Incubate overnight in a 4°C refrigerator.

(4) Antibody incubation: After the serum was incubated overnight, the plate was washed 4 times. The HRP-labeled antibody was diluted to the corresponding multiples with antibody diluent, 100 μ L of antibody diluent was added to each well and incubated at 25°C for 2 h.

(5) Color development and detection: After the antibody incubation, wash the plate 4 times. Add 100 μ L of chromogenic solution to each well and incubate at 25°C for 30 min in the dark. Add 100 μ L of sulfuric acid stop solution to each well. Immediately after adding the stop solution, read the absorbance values at 450 nm and 630 nm with a microplate reader. Analyze the data, and determine the dilution end point according to the difference between the absorbance values of the Control group, so as to determine the dilution factor of the end point in the immunization group, which is the titer. RBD of each group of II-5 LNP@S mRNA, II-18 LNP@S mRNA, II-24 LNP@S mRNA, II-25 LNP@S mRNA, III-3 LNP@S mRNA and III-11 LNP@S mRNA The specific IgG titer is shown in Figure 9, and the results show that the ionizable lipids of the present disclosure can induce strong humoral immune responses when used in SARS-CoV-2 mRNA vaccines.

Example 36 Safety evaluation of LNP@S mRNA vaccines

(1) Preparation and administration of preparations: II-5 LNP@S mRNA, II-18 LNP@S mRNA, II-24 LNP@S mRNA, II-25 LNP@S mRNA, III-3 LNP @S mRNA and III-11 LNP@S mRNA preparations were prepared according to Example 33. The mRNA concentration of the adjusted preparation was 0.1 mg/mL, and the blank BALB/c mice were intramuscularly injected with 200 μ L, that is, 20 μ g S mRNA/mice, and an equal volume of PBS was used as a control.

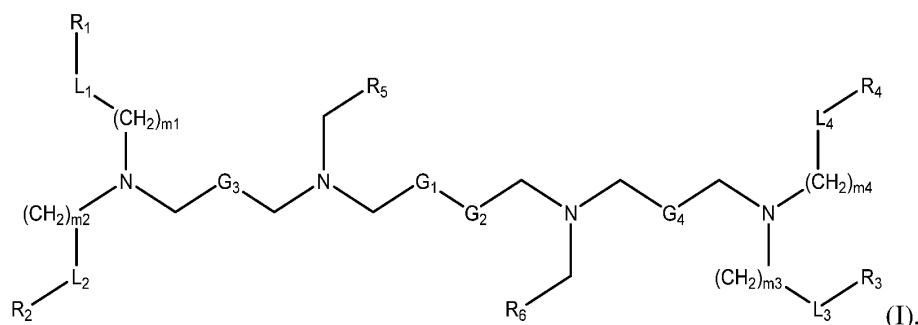
(2) Blood collection and detection: 24 hours after administration, the orbital blood of mice

was collected, centrifuged, and the serum was drawn to use a biochemical analyzer to detect major biochemical indicators, as shown in Figures 10-12. The results show that the ionizable lipids of the present disclosure are safe when used in SARS-CoV-2 mRNA vaccines.

Notably, the specific features, structures, materials or features described in this specification may be combined in an appropriate manner in any one or more examples. In addition, technicians in the field may combine the different examples described in this specification and the characteristics of different embodiments under non-contradictory condition.

CLAIMS

1. A compound of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein

m_1 , m_2 , m_3 , and m_4 are each independently selected from 1, 2, 3, 4, or 5;

L_1 , L_2 , L_3 , and L_4 are each independently selected from $-\text{CH}(\text{OH})-$, $-\text{C}(=\text{O})-$, $-\text{C}(=\text{O})\text{O}-$, $-\text{OC}(=\text{O})-$, $-\text{C}(=\text{O})\text{S}-$, $-\text{SC}(=\text{O})-$, $-\text{C}(=\text{O})\text{NR}_a-$, $-\text{NR}_a\text{C}(=\text{O})-$, $-\text{NR}_a\text{C}(=\text{O})\text{O}-$, $-\text{OC}(=\text{O})\text{NR}_a-$, $-\text{O}-$, $-\text{O}-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{S}-\text{S}-\text{S}-$, $-\text{CH}(\text{OH})\text{CH}_2\text{O}-$, $-\text{CH}(\text{OH})\text{CH}_2\text{S}-$, or absent, wherein

each R_a is independently $-\text{H}$ or optionally substituted C_1 - C_6 alkyl;

R_1 , R_2 , R_3 , and R_4 are each independently selected from optionally substituted C_6 - C_{30} alkyl, optionally substituted C_6 - C_{30} alkenyl, or optionally substituted C_6 - C_{30} alkynyl;

G_1 , G_2 , G_3 and G_4 are each independently selected from $-\text{R}_c-$, $-\text{R}_c\text{CH}(\text{OH})\text{R}_d-$, $-\text{R}_c\text{C}(=\text{O})\text{R}_d-$, $-\text{R}_c\text{C}(=\text{O})\text{OR}_d-$, $-\text{R}_c\text{OC}(=\text{O})\text{R}_d-$, $-\text{R}_c\text{C}(=\text{O})\text{SR}_d-$, $-\text{R}_c\text{SC}(=\text{O})\text{R}_d-$, $-\text{R}_c\text{C}(=\text{O})\text{N}(\text{R}_b)\text{R}_d-$, $-\text{R}_c\text{N}(\text{R}_b)\text{C}(=\text{O})\text{R}_d-$, $-\text{R}_c\text{N}(\text{R}_b)\text{C}(=\text{O})\text{OR}_d-$, $-\text{R}_c\text{OC}(=\text{O})\text{N}(\text{R}_b)\text{R}_d-$, $-\text{R}_c\text{OR}_d-$, $-\text{R}_c-\text{O}-\text{O}-\text{R}_d-$, $-\text{R}_c\text{SR}_d-$, $-\text{R}_c-\text{S}-\text{S}-\text{R}_d-$, $-\text{R}_c-\text{S}-\text{S}-\text{S}-\text{R}_d-$, or absent; wherein

each R_b is independently $-\text{H}$ or optionally substituted C_1 - C_6 alkyl;

each R_c and R_d are independently $-(\text{CH}_2)_n-$, and n is 0, 1, 2, 3, or 4;

R_5 and R_6 are each independently selected from $-\text{H}$, $-\text{OH}$, or optionally substituted C_1 - C_6 alkyl.

2. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein

G_1 and G_2 are each independently $-\text{R}_c-$, G_3 and G_4 are each independently selected from $-\text{R}_c-$, $-\text{R}_c\text{C}(=\text{O})\text{R}_d-$, $-\text{R}_c\text{C}(=\text{O})\text{OR}_d-$, $-\text{R}_c\text{OC}(=\text{O})\text{R}_d-$, $-\text{R}_c\text{C}(=\text{O})\text{N}(\text{R}_b)\text{R}_d-$, $-\text{R}_c\text{N}(\text{R}_b)\text{C}(=\text{O})\text{R}_d-$, $-\text{R}_c\text{N}(\text{R}_b)\text{C}(=\text{O})\text{OR}_d-$, $-\text{R}_c\text{OC}(=\text{O})\text{N}(\text{R}_b)\text{R}_d-$, or absent;

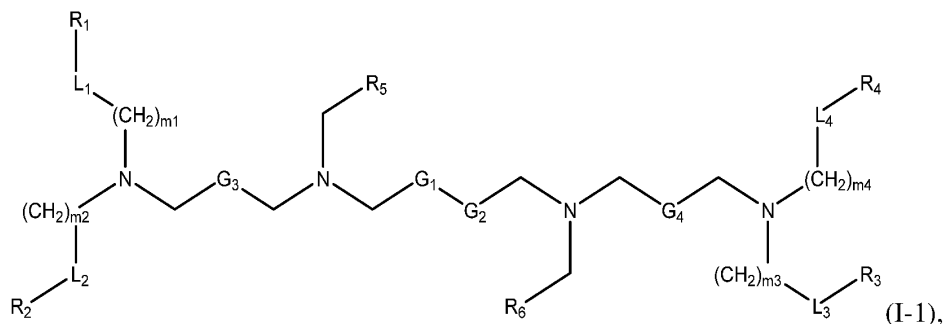
each R_b is independently $-\text{H}$ or C_1 - C_6 alkyl;

each R_c and R_d are independently $-(CH_2)_n-$, and n is 0, 1, 2, 3, or 4.

3. The compound of claim 2 or a pharmaceutically acceptable salt thereof, wherein
 G_1 and G_2 are each independently $-R_c-$, G_3 and G_4 are each independently selected from $-R_c-$, $-R_cC(=O)OR_d-$, $-R_cOC(=O)R_d-$, $-R_cC(=O)N(R_b)R_d-$, $-R_cN(R_b)C(=O)R_d-$, or absent;
each R_b is independently $-H$ or C_1-C_2 alkyl;
each R_c and R_d are independently $-(CH_2)_n-$ or absent, and n is 0, 1 or 2.
4. The compound of claim 3 or a pharmaceutically acceptable salt thereof, wherein
 G_1 and G_2 are absent, G_3 and G_4 are each independently selected from $-CH_2-$, $-CH_2C(=O)OCH_2-$, $-CH_2OC(=O)CH_2-$, $-CH_2C(=O)NHCH_2-$, $-CH_2NHC(=O)CH_2-$, or absent.
5. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein
 G_3 and G_4 are each independently $-R_c-$, G_1 and G_2 are each independently selected from $-R_c-$, $-R_cC(=O)R_d-$, $-R_cC(=O)OR_d-$, $-R_cOC(=O)R_d-$, $-R_cC(=O)N(R_b)R_d-$, $-R_cN(R_b)C(=O)R_d-$, $-R_cN(R_b)C(=O)OR_d-$, $-R_cOC(=O)N(R_b)R_d-$, or absent;
each R_b is independently $-H$ or C_1-C_6 alkyl;
each R_c and R_d are independently $-(CH_2)_n-$, and n is 0, 1, 2, 3, or 4.
6. The compound of claim 5 or a pharmaceutically acceptable salt thereof, wherein
 G_3 and G_4 are each independently $-R_c-$, G_1 and G_2 are each independently selected from $-R_c-$, $-R_cC(=O)OR_d-$, $-R_cOC(=O)R_d-$, $-R_cC(=O)N(R_b)R_d-$, $-R_cN(R_b)C(=O)R_d-$, or absent;
each R_b is independently $-H$ or C_1-C_2 alkyl;
each R_c and R_d are independently $-(CH_2)_n-$ or absent, and n is 0, 1 or 2.
7. The compound of claim 6 or a pharmaceutically acceptable salt thereof, wherein
 G_3 and G_4 are absent, G_1 and G_2 are each independently selected from $-CH_2-$, $-CH_2C(=O)O(CH_2)_{1 \text{ or } 2}-$, $-(CH_2)_{1 \text{ or } 2}OC(=O)CH_2-$, $-CH_2C(=O)N(R_b)CH_2-$, $-CH_2N(R_b)C(=O)CH_2-$, or absent;
each R_b is independently $-H$ or C_1-C_2 alkyl.

8. The compound of any one of claims 1-7 or a pharmaceutically acceptable salt thereof, wherein L_1 , L_2 , L_3 , and L_4 are each independently selected from $-\text{CH}(\text{OH})-$, $-\text{C}(=\text{O})-$, $-\text{C}(=\text{O})\text{O}-$, $-\text{OC}(=\text{O})-$, $-\text{C}(=\text{O})\text{NR}_a-$, $-\text{NR}_a\text{C}(=\text{O})-$, $-\text{NR}_a\text{C}(=\text{O})\text{O}-$, $-\text{OC}(=\text{O})\text{NR}_a-$, $-\text{O}-$, $-\text{S}-$, $-\text{CH}(\text{OH})\text{CH}_2\text{O}-$, $-\text{CH}(\text{OH})\text{CH}_2\text{S}-$, or absent.
9. The compound of any one of claims 1-7 or a pharmaceutically acceptable salt thereof, wherein L_1 , L_2 , L_3 , and L_4 are each independently selected from $-\text{CH}(\text{OH})-$, $-\text{C}(=\text{O})-$, $-\text{C}(=\text{O})\text{O}-$, $-\text{OC}(=\text{O})-$, $-\text{C}(=\text{O})\text{NR}_a-$, $-\text{NR}_a\text{C}(=\text{O})-$, $-\text{O}-$, $-\text{S}-$, or absent; each R_a is independently $-\text{H}$ or $\text{C}_1\text{-C}_2$ alkyl.
10. The compound of any one of claims 1-9 or a pharmaceutically acceptable salt thereof, wherein m_1 , m_2 , m_3 , and m_4 are each independently selected from 1 or 2.
11. The compound of any one of claims 1-10 or a pharmaceutically acceptable salt thereof, wherein R_1 , R_2 , R_3 , and R_4 are each independently selected from $\text{C}_6\text{-C}_{18}$ alkyl, $\text{C}_6\text{-C}_{18}$ alkenyl, or $\text{C}_6\text{-C}_{18}$ alkynyl, wherein said $\text{C}_6\text{-C}_{18}$ alkyl, $\text{C}_6\text{-C}_{18}$ alkenyl, or $\text{C}_6\text{-C}_{18}$ alkynyl is optionally substituted one to three groups selected from halogen, OH , or $=\text{O}$.
12. The compound of any one of claims 1-10 or a pharmaceutically acceptable salt thereof, wherein R_1 , R_2 , R_3 , and R_4 are each independently selected from $\text{C}_6\text{-C}_{18}$ alkyl.
13. The compound of any one of claims 1-12 or a pharmaceutically acceptable salt thereof, wherein R_5 and R_6 are each independently selected from $-\text{H}$, $-\text{OH}$, or $\text{C}_1\text{-C}_4$ alkyl optionally substituted with $-\text{OH}$.

14. A compound of Formula (I-1):



or a pharmaceutically acceptable salt thereof, wherein

m_1 , m_2 , m_3 , and m_4 are the same, all of which are 1, 2, 3, 4, or 5;

L_1 , L_2 , L_3 , and L_4 are the same, all of which are selected from $-\text{CH}(\text{OH})-$, $-\text{C}(=\text{O})-$, $^-\text{C}(=\text{O})\text{O}-^{\wedge\wedge}$, $^-\text{OC}(=\text{O})-^{\wedge\wedge}$, $^-\text{C}(=\text{O})\text{S}-^{\wedge\wedge}$, $^-\text{SC}(=\text{O})-^{\wedge\wedge}$, $^-\text{C}(=\text{O})\text{NR}_a-^{\wedge\wedge}$, $^-\text{NR}_a\text{C}(=\text{O})-^{\wedge\wedge}$, $^-\text{NR}_a\text{C}(=\text{O})\text{O}-^{\wedge\wedge}$, $^-\text{OC}(=\text{O})\text{NR}_a-^{\wedge\wedge}$, $-\text{O}-$, $-\text{O}-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{S}-\text{S}-\text{S}-$, $^-\text{CH}(\text{OH})\text{CH}_2\text{O}-^{\wedge\wedge}$, $^-\text{CH}(\text{OH})\text{CH}_2\text{S}-^{\wedge\wedge}$ or absent, wherein

$^{\wedge\wedge}$ represents the point which attaches to R_{1-4} ; $-\wedge\wedge$ represents the point which attaches to $-(\text{CH}_2)_{m_1-m_4}-$; and

each R_a is independently $-\text{H}$ or optionally substituted C_1 - C_6 alkyl;

R_1 , R_2 , R_3 , and R_4 are the same, all of which are selected from optionally substituted C_6 - C_{30} alkyl, optionally substituted C_6 - C_{30} alkenyl, or optionally substituted C_6 - C_{30} alkynyl;

G_1 and G_2 are the same, and G_3 and G_4 are the same,

when G_1 and G_2 are $-\text{R}_c-$, G_3 and G_4 are selected from $-\text{R}_c-$,

$^-\text{R}_c\text{CH}(\text{OH})\text{R}_d-^{**}$, $^-\text{R}_c\text{C}(=\text{O})\text{R}_d-^{**}$, $^-\text{R}_c\text{C}(=\text{O})\text{OR}_d-^{**}$, $^-\text{R}_c\text{OC}(=\text{O})\text{R}_d-^{**}$, $^-\text{R}_c\text{C}(=\text{O})\text{SR}_d-^{**}$, $^-\text{R}_c\text{SC}(=\text{O})\text{R}_d-^{**}$, $^-\text{R}_c\text{C}(=\text{O})\text{N}(\text{R}_b)\text{R}_d-^{**}$, $^-\text{R}_c\text{N}(\text{R}_b)\text{C}(=\text{O})\text{R}_d-^{**}$, $^-\text{R}_c\text{N}(\text{R}_b)\text{C}(=\text{O})\text{OR}_d-^{**}$, $^-\text{R}_c\text{OC}(=\text{O})\text{N}(\text{R}_b)\text{R}_d-^{**}$, $^-\text{R}_c\text{OR}_d-^{**}$, $^-\text{R}_c-\text{O}-\text{O}-\text{R}_d-^{**}$, $^-\text{R}_c\text{SR}_d-^{**}$, $^{**}-\text{R}_c-\text{S}-\text{S}-\text{R}_d-^{**}$, $^-\text{R}_c-\text{S}-\text{S}-\text{S}-\text{R}_d-^{**}$, or absent; wherein

$^*-$ represents the point which attaches to the $-\text{CH}_2-$ group next to the terminal tertiary amine atom as shown in Formula (I); ** represents the point which attaches to the $-\text{CH}_2-$ group next to the middle tertiary amine atom as shown in Formula (I);

when G_3 and G_4 are $-\text{R}_c-$, G_1 and G_2 are selected from $-\text{R}_c-$, $^{\#}-\text{R}_c\text{CH}(\text{OH})\text{R}_d-^{\#\#}$, $^{\#}-\text{R}_c\text{C}(=\text{O})\text{R}_d-^{\#\#}$, $^{\#}-\text{R}_c\text{C}(=\text{O})\text{OR}_d-^{\#\#}$, $^{\#}-\text{R}_c\text{OC}(=\text{O})\text{R}_d-^{\#\#}$, $^{\#}-\text{R}_c\text{C}(=\text{O})\text{SR}_d-^{\#\#}$,

$\#-R_cSC(=O)R_d-^{\#\#}$, $\#-R_cC(=O)N(R_b)R_d-^{\#\#}$, $\#-R_cN(R_b)C(=O)R_d-^{\#\#}$,
 $\#-R_cN(R_b)C(=O)OR_d-^{\#\#}$, $\#-R_cOC(=O)N(R_b)R_d-^{\#\#}$, $\#-R_cOR_d-^{\#\#}$, $\#-R_c-O-O-R_d-^{\#\#}$,
 $\#-R_cSR_d-^{\#\#}$, $\#-R_c-S-S-R_d-^{\#\#}$, $\#-R_c-S-S-S-R_d-^{\#\#}$, or absent;

wherein

$\#$ - represents the point which attaches to the $-CH_2-$ group next to the middle tertiary amine atom as shown in Formula (I); $-^{\#\#}$ represents the point connecting G_1 and G_2 ; and

each R_b is independently $-H$ or optionally substituted C_1-C_6 alkyl;

each R_c and R_d are independently $-(CH_2)_n-$, and n is 0, 1, 2, 3, or 4;

R_5 and R_6 are the same, both of which are selected from $-H$, $-OH$, or optionally substituted C_1-C_6 alkyl.

15. The compound of claim 14 or a pharmaceutically acceptable salt thereof, wherein

G_1 and G_2 are $-R_c-$, G_3 and G_4 are selected from $-R_c-$, $*-R_cC(=O)R_d-^{**}$,
 $*-R_cC(=O)OR_d-^{**}$, $*-R_cOC(=O)R_d-^{**}$, $*-R_cC(=O)N(R_b)R_d-^{**}$,
 $*-R_cN(R_b)C(=O)R_d-^{**}$, $*-R_cN(R_b)C(=O)OR_d-^{**}$, $*-R_cOC(=O)N(R_b)R_d-^{**}$, or absent;

each R_b is independently $-H$ or C_1-C_6 alkyl;

each R_c and R_d are independently $-(CH_2)_n-$, and n is 0, 1, 2, 3, or 4.

16. The compound of claim 15 or a pharmaceutically acceptable salt thereof, wherein

G_1 and G_2 are $-R_c-$, G_3 and G_4 are selected from $*-R_cC(=O)OR_d-^{**}$,
 $*-R_cOC(=O)R_d-^{**}$, $*-R_cC(=O)N(R_b)R_d-^{**}$, $*-R_cN(R_b)C(=O)R_d-^{**}$, or absent;

each R_b is independently $-H$ or C_1-C_2 alkyl;

each R_c and R_d are independently $-(CH_2)_n-$ or absent, and n is 0, 1 or 2.

17. The compound of claim 16 or a pharmaceutically acceptable salt thereof, wherein

G_1 and G_2 are absent, G_3 and G_4 are selected from $-CH_2-$,
 $*-CH_2C(=O)OCH_2-^{**}$, $*-CH_2OC(=O)CH_2-^{**}$, $*-CH_2C(=O)NHCH_2-^{**}$,
 $*-CH_2NHC(=O)CH_2-^{**}$, or absent.

18. The compound of claim 14 or a pharmaceutically acceptable salt thereof, wherein
 G_3 and G_4 are $-R_c-$, G_1 and G_2 are selected from $-R_c-$, $^{\#}-R_cC(=O)R_d-^{\#\#}$,
 $^{\#}-R_cC(=O)OR_d-^{\#\#}$, $^{\#}-R_cOC(=O)R_d-^{\#\#}$, $^{\#}-R_cC(=O)N(R_b)R_d-^{\#\#}$, $^{\#}-R_cN(R_b)C(=O)R_d-^{\#\#}$,
 $^{\#}-R_cN(R_b)C(=O)OR_d-^{\#\#}$, $^{\#}-R_cOC(=O)N(R_b)R_d-^{\#\#}$, or absent;
each R_b is independently $-H$ or C_1-C_6 alkyl;
each R_c and R_d are independently $-(CH_2)_n-$, and n is 0, 1, 2, 3, or 4.
19. The compound of claim 18 or a pharmaceutically acceptable salt thereof, wherein
 G_3 and G_4 are $-R_c-$, G_1 and G_2 are selected from $-R_c-$, $^{\#}-R_cC(=O)OR_d-^{\#\#}$,
 $^{\#}-R_cOC(=O)R_d-^{\#\#}$, $^{\#}-R_cC(=O)N(R_b)R_d-^{\#\#}$, $^{\#}-R_cN(R_b)C(=O)R_d-^{\#\#}$, or absent;
each R_b is independently $-H$ or C_1-C_2 alkyl;
each R_c and R_d are independently $-(CH_2)_n-$ or absent, and n is 0, 1 or 2.
20. The compound of claim 19 or a pharmaceutically acceptable salt thereof, wherein
 G_3 and G_4 are absent, G_1 and G_2 are selected from $-CH_2-$,
 $^{\#}-CH_2C(=O)O(CH_2)_{1 \text{ or } 2}-^{\#\#}$, $^{\#}-(CH_2)_{1 \text{ or } 2}OC(=O)CH_2-^{\#\#}$, $^{\#}-CH_2C(=O)N(R_b)CH_2-^{\#\#}$,
 $^{\#}-CH_2N(R_b)C(=O)CH_2-^{\#\#}$, or absent;
each R_b is independently $-H$ or C_1-C_2 alkyl.
21. The compound of any one of claims 14-20 or a pharmaceutically acceptable salt thereof, wherein L_1 , L_2 , L_3 , and L_4 are the same, all of which are selected from
 $-CH(OH)-$, $-C(=O)-$, $^{\wedge}-C(=O)O-^{\wedge\wedge}$, $^{\wedge}-OC(=O)-^{\wedge\wedge}$, $^{\wedge}-C(=O)NR_a-^{\wedge\wedge}$, $^{\wedge}-NR_aC(=O)-^{\wedge\wedge}$,
 $^{\wedge}-NR_aC(=O)O-^{\wedge\wedge}$, $^{\wedge}-OC(=O)NR_a-^{\wedge\wedge}$, $-O-$, $-S-$, $^{\wedge}-CH(OH)CH_2O-^{\wedge\wedge}$,
 $^{\wedge}-CH(OH)CH_2S-^{\wedge\wedge}$, or absent.
22. The compound of any one of claims 14-20 or a pharmaceutically acceptable salt thereof, wherein L_1 , L_2 , L_3 , and L_4 are the same, all of which are selected from
 $-CH(OH)-$, $-C(=O)-$, $^{\wedge}-C(=O)O-^{\wedge\wedge}$, $^{\wedge}-OC(=O)-^{\wedge\wedge}$, $^{\wedge}-C(=O)NR_a-^{\wedge\wedge}$, $^{\wedge}-NR_aC(=O)-^{\wedge\wedge}$,
 $-O-$, $-S-$, or absent; each R_a is independently $-H$ or C_1-C_2 alkyl.
23. The compound of any one of claims 14-22 or a pharmaceutically acceptable salt thereof, wherein m_1 , m_2 , m_3 , and m_4 are the same, all of which are 1 or 2.

24. The compound of any one of claims 14-23 or a pharmaceutically acceptable salt thereof, wherein R_1 , R_2 , R_3 , and R_4 are the same, all of which are selected from C_6 - C_{18} alkyl, C_6 - C_{18} alkenyl, or C_6 - C_{18} alkynyl, wherein said C_6 - C_{18} alkyl, C_6 - C_{18} alkenyl, or C_6 - C_{18} alkynyl is optionally substituted one to three groups selected from halogen, OH, or =O.
25. The compound of any one of claims 14-23 or a pharmaceutically acceptable salt thereof, wherein R_1 , R_2 , R_3 , and R_4 are the same, all of which are selected from C_6 - C_{18} alkyl.
26. The compound of any one of claims 14-25 or a pharmaceutically acceptable salt thereof, wherein R_5 and R_6 are the same, both of which are selected from -H, -OH, or C_1 - C_4 alkyl optionally substituted with -OH.
27. A compound of Table 1, or a pharmaceutically acceptable salt thereof.
28. The compound of any one of claims 1-27, wherein the compound is in the form of a pharmaceutically acceptable salt.
29. A pharmaceutical composition comprising the compound of any one of claims 1-27, or a pharmaceutically acceptable salt thereof, and a nucleic acid drug.
30. The pharmaceutical composition of claim 29, wherein the composition further comprises at least one excipient selected from a neutral phospholipid, a steroid, and a polyethylene glycol lipid.
31. The pharmaceutical composition of claim 30, wherein the neutral phospholipid is selected from DOPE, DSPC, DOPC, DSPE, DMPC, DMPE, DPPC, DPPE, DEPC, HSPC, POPC or a combination thereof.
32. The pharmaceutical composition of claim 31, wherein the neutral phospholipid is DOPE.

33. The pharmaceutical composition of claim 30, wherein the mole ratio of the compound : the neutral phospholipid is 1:1~ 5:1.
34. The pharmaceutical composition of any one of claims 30-33, wherein the steroid is selected from cholesterol, sitosterol, soybean sterol, wool sterol, ergosterol or a combination thereof.
35. The pharmaceutical composition of claim 34, wherein the steroid is cholesterol.
36. The pharmaceutical composition of any one of claims 30-35, wherein the mole ratio of the compound: the steroid is 1:2 ~ 2:1.
37. The pharmaceutical composition of any one of claims 30-36, wherein the polyethylene glycol lipid is selected from DMG-PEG, DSPE-PEG or a combination thereof.
38. The pharmaceutical composition of any one of claims 30-36, wherein the polyethylene glycol lipid is DMG-PEG2000.
39. The pharmaceutical composition of any one of claims 30-38, wherein the mole ratio of the compound: the polyethylene glycol lipid is 5:1~100:1.
40. The pharmaceutical composition of any one of claims 30-39, wherein the mole ratio of the compound: the polyethylene glycol lipid is 10:1 ~ 20:1.
41. The pharmaceutical composition of any one of claims 30-40, wherein the nucleic acid drug is selected from DNA, ASO, siRNA, miRNA, mRNA, ribozyme, aptamer or a combination thereof, preferably, mRNA.
42. The pharmaceutical composition of any one of claims 30-41, wherein the pharmaceutical composition is prepared into lipid nanoparticles (LNP).