DELIVERY COMPOSITIONS

RELATED APPLICATIONS

This application claims priority to U.S. Serial No.: 63/552,586, filed February 12, 2024; U.S. Serial No.: 63/666,081, filed June 28, 2024; and U.S. Serial No.: 63/725,955, filed Nov 27, 2024, the entire contents of each of which are incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on February 11, 2025, is named A2002-7000TW_SL.xml and is 129,622 bytes in size.

FIELD OF THE INVENTION

The invention provides novel ionizable lipids and lipid nanoparticles for the delivery of therapeutic nucleic acids (e.g., siRNA, mRNA, gRNA) both *in vitro* and *in vivo*.

BACKGROUND

Efficient targeted delivery of nucleic acids to cells is difficult due to the relative instability and low cell permeability of these species. Therefore, there is a need to develop methods and compositions that facilitate the delivery of therapeutic and/or prophylactic agents, such as nucleic acids, to cells. Preferably, these lipid-nucleic acid particles should be well-tolerated and provide an adequate therapeutic index, such that patient treatment at an effective dose of the nucleic acid is not associated with unacceptable toxicity and/or risk to the patient. The present invention provides these and related advantages.

SUMMARY

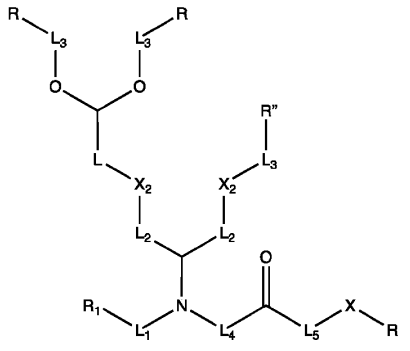
The present invention recognizes a need for compositions, preparations, nanoparticles, and/or nanomaterials and methods of their use. Among other things, the present disclosure recognizes that structural features of compositions, preparations, nanoparticles, and/or nanomaterials impact functional responses *in vivo*, *in vitro*, and *ex vivo*. For example, the present disclosure describes,

among other things, that selection and combination of one or more components described herein influence functional activity of lipid nanoparticles. In some embodiments, for example, functional activity can refer to desired tropisms, stabilization, and/or drug delivery efficacy. In some embodiments, among other things, the present disclosure describes that different ratios of one of more components influence one or more functional activities of compositions, preparations, nanoparticles, and/or nanomaterials described herein.

The lipid nanoparticle compositions provided herein may further comprise a nucleic acid, such as an RNA, e.g., a messenger RNA (mRNA). The lipid nanoparticle compositions may be used for mRNA delivery to a cell in a subject. Messenger RNA based gene therapy requires efficient delivery of mRNA to circulating cells (e.g., immune cells) in plasma or to cells in a given tissue. The main challenges associated with efficient mRNA delivery to attain robust levels of protein expression include: (a) ability to protect the mRNA payload against prevalent serum nucleases upon administration to a subject; (b) the ability to specifically target mRNA delivery to, and thereby maximize protein expression in the target cell population; and (c) the ability to maximally deliver the mRNA payload to the cytosolic compartment of cells for translation into proteins within the cytoplasm.

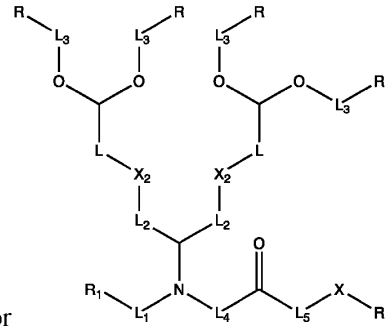
The invention provides ionizable cationic lipids for producing lipid nanoparticle compositions that facilitate the delivery of a payload (e.g., a nucleic acid) disposed therein to cells, for example, mammalian cells, for example, immune cells. The lipids are designed to enable intracellular delivery of a nucleic acid, e.g., mRNA, to the cytosolic compartment of a target cell type and rapidly degrade into non-toxic components. Without wishing to be bound by theory, in some embodiments these complex functionalities are achieved by the interplay between chemistry and geometry of the ionizable lipid head group, the hydrophobic "acyl-tail" groups and the linker connecting the head group and the acyl tail groups in the ionizable cationic lipids.

In one aspect, the present invention provides a compound represented by Formula (A-I) or Formula (A-II):



(A-I)

or



(A-II)

or its N-oxide, isomer, or a pharmaceutically acceptable salt thereof, wherein

L is optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

L_1 is optionally substituted C_{1-6} alkylenyl, or C_{2-6} heteroalkylenyl;

each L_2 is independently C_{1-6} alkylenyl;

L_3 is optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

L_4 is absent, optionally substituted C_{1-10} alkylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

L_5 is optionally substituted C_{1-10} alkyl, optionally substituted C_{1-10} alkenyl, optionally substituted C_{1-10} alkynyl, or optionally substituted C_{2-10} heteroalkylenyl;

X is absent, -OC(O)-, -C(O)O-, -NHC(O)-, -C(O)NH-, or -OC(O)O-;

each X_2 is absent, -OC(O)-, -C(O)O-, -NHC(O)-, -C(O)NH-, or -OC(O)O-;

each of R is independently hydrogen, OR_6, or an optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

R_1 is hydrogen, optionally substituted phenyl, optionally substituted 3- to 7-membered cycloaliphatic, optionally substituted 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 8- to 10-membered bicyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, $-OR_2$, $-C(O)OR_2$, $-C(O)SR_2$, $-OC(O)R_2$, $-OC(O)OR_2$, $-CN$, $-N(R_2)_2$, $-C(O)N(R_2)_2$, $-S(O)_2N(R_2)_2$, $-NR_2C(O)R_2$, $-OC(O)N(R_2)_2$, $-N(R_2)C(O)OR_2$, $-NR_2S(O)_2R_2$, $-NR_2C(O)N(R_2)_2$, $-NR_2C(S)N(R_2)_2$, $-NR_2C(NR_2)N(R_2)_2$, $-NR_2C(CHR_2)N(R_2)_2$, $-N(OR_2)C(O)R_2$, $-N(OR_2)S(O)_2R_2$, $-N(OR_2)C(O)OR_2$, $-N(OR_2)C(O)N(R_2)_2$, $-N(OR_2)C(S)N(R_2)_2$, $-N(OR_2)C(NR_2)N(R_2)_2$, $-N(OR_2)C(CHR_2)N(R_2)_2$, $-C(NR_2)N(R_2)_2$, $-C(NR_2)R_2$, $-C(O)N(R_2)OR_2$, $-C(R_2)N(R_2)_2C(O)OR_2$, $-CR_2(R_3)_2$, $-OP(O)(OR_2)_2$, or $-P(O)(OR_2)_2$; or
R_1 is a ring selected from 3- to 7-membered cycloaliphatic and 3- to 7- membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, wherein the cycloaliphatic or heterocyclyl ring is optionally substituted with 1-4 R_2 or R_3 groups;

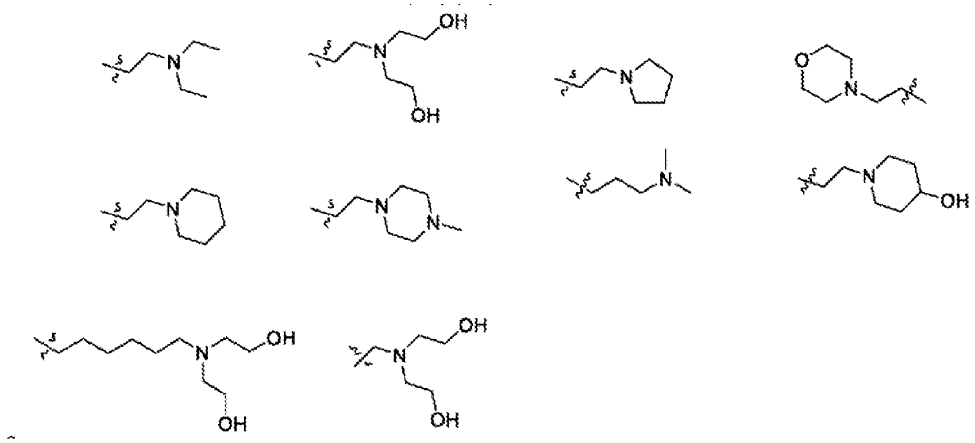
each R_2 is independently hydrogen, oxo, $-CN$, $-NO_2$, $-OR_4$, $-S(O)_2R_4$, $-S(O)_2N(R_4)_2$, $-(CH_2)_n-R_4$, or an optionally substituted group selected from C_{1-6} aliphatic, phenyl, 3- to 7-membered cycloaliphatic, 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; or two occurrences of R_2, taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur; each R_3 is independently $-(CH_2)n-R_4$; or two occurrences of R_3, taken together with the atom(s) to which they are attached, form optionally substituted 5- to 6-

membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur; each R_4 is independently hydrogen, $-OR_5$, $-N(R_5)_2$, $-OC(O)R_5$, $-OC(O)OR_5$, -CN, $-C(O)N(R_5)_2$, $-NR_5C(O)R_5$, $-OC(O)N(R_5)_2$, $-N(R_5)C(O)OR_5$, $-NR_5S(O)_2R_5$, $-NR_5C(O)N(R_5)_2$, $-NR_5C(S)N(R_5)_2$, or $-NR_5C(NR_5)N(R_5)_2$; and

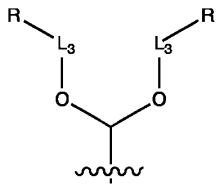
each R_5 is independently hydrogen, or optionally substituted C_{1-6} aliphatic; or two occurrences of R_5, taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur; each R_6 is independently C_{4-12} aliphatic; and each n is independently 0 to 4.

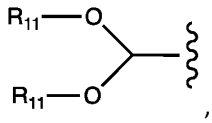
In some embodiments, R_1 is $-N(R_2)_2$.

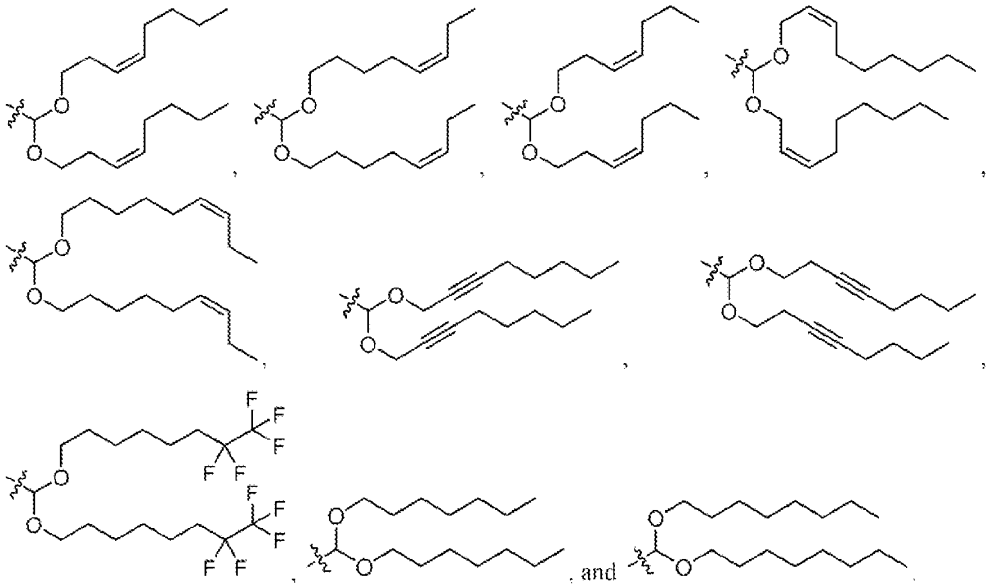
In some embodiments, L_1-R_1 is selected from the group consisting of

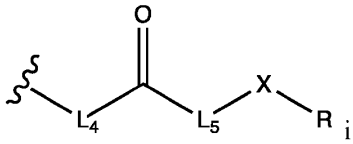
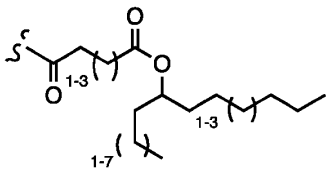


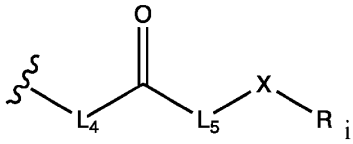
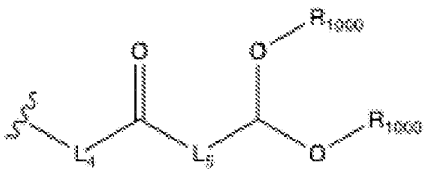
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.In some embodiments,  is selected from the group consisting of:

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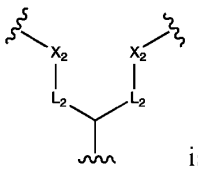
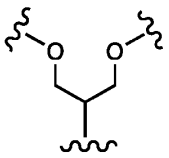
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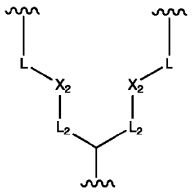
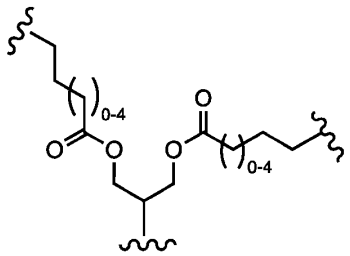
In some embodiments,  is .

In some embodiments,  is 

where R_{1000} is optionally substituted C_{1-10} alkyl, optionally substituted C_{2-10} alkenyl, optionally

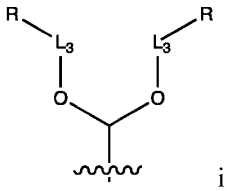
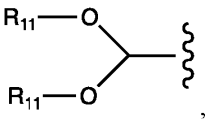
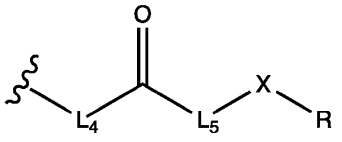
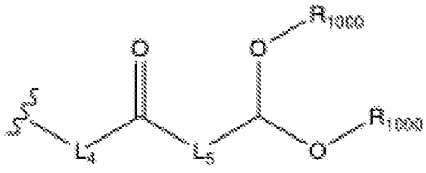
substituted C_{2-10} alkynyl, optionally substituted C_{3-10} cyclooalkyl or optionally substituted C_{5-10} heteroalkyl.

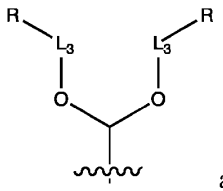
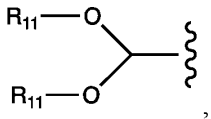
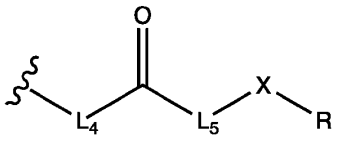
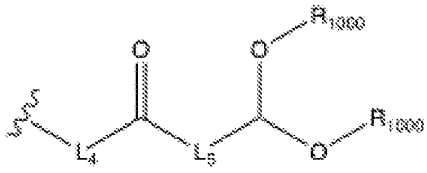
In some embodiments,  is .

In some embodiments,  is .

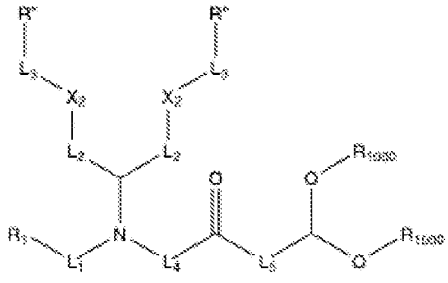
In some embodiments, x is -OC(O)-, -C(O)O-, -NHC(O)-, -C(O)NH-, or -OC(O)O-.

In some embodiments, x is -OC(O)-.

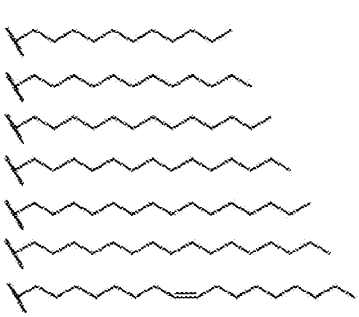
In some embodiments, when at least one  is not , then  is  where R_{1000} is optionally substituted C_{1-10} alkyl, optionally substituted C_{2-10} alkenyl, optionally substituted C_{2-10} alkynyl, optionally substituted C_{3-10} cyclooalkyl or optionally substituted C_{5-10} heteroalkyl.

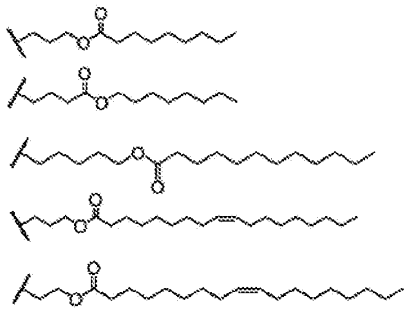
In some embodiments, when both  are not , then  is  where R_{1000} is optionally substituted C_{1-10} alkyl, optionally substituted C_{2-10} alkenyl, optionally substituted C_{2-10} alkynyl, optionally substituted C_{3-10} cyclooalkyl or optionally substituted C_{5-10} heteroalkyl.

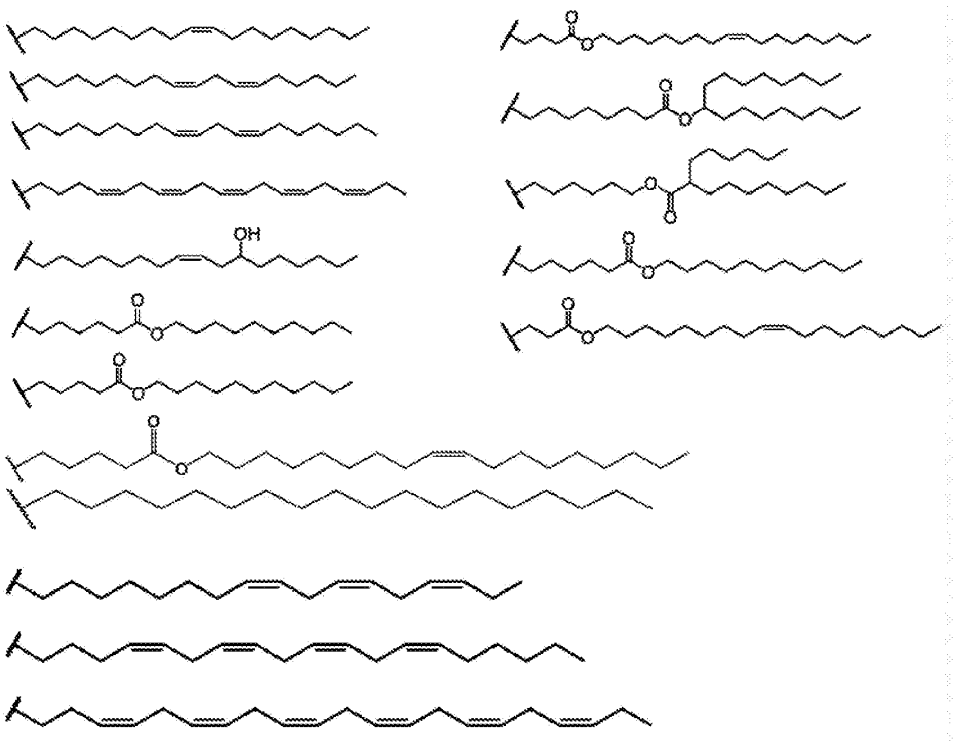
In some embodiments, a compound of formula (A-III)

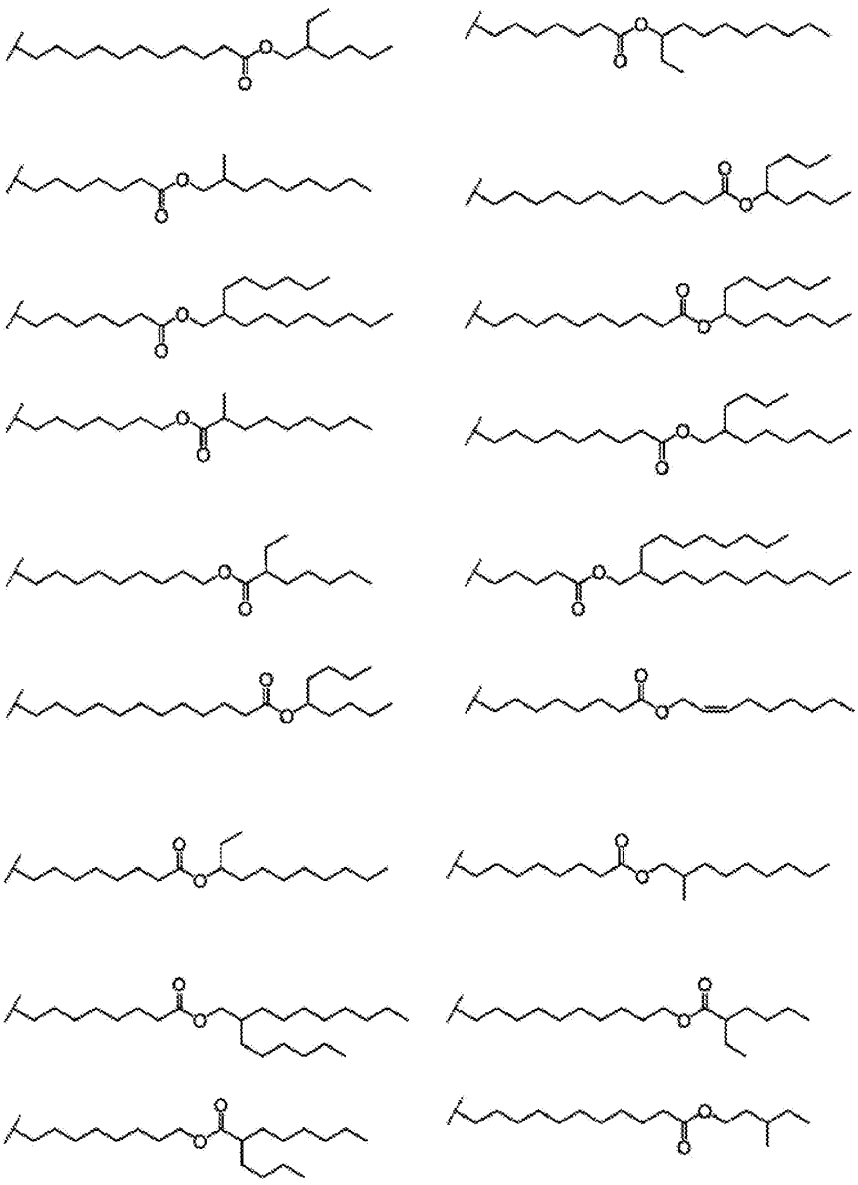
 (A-III); where L_1-L_5, X_2, R_1, R" and R_{1000} are as previously defined.

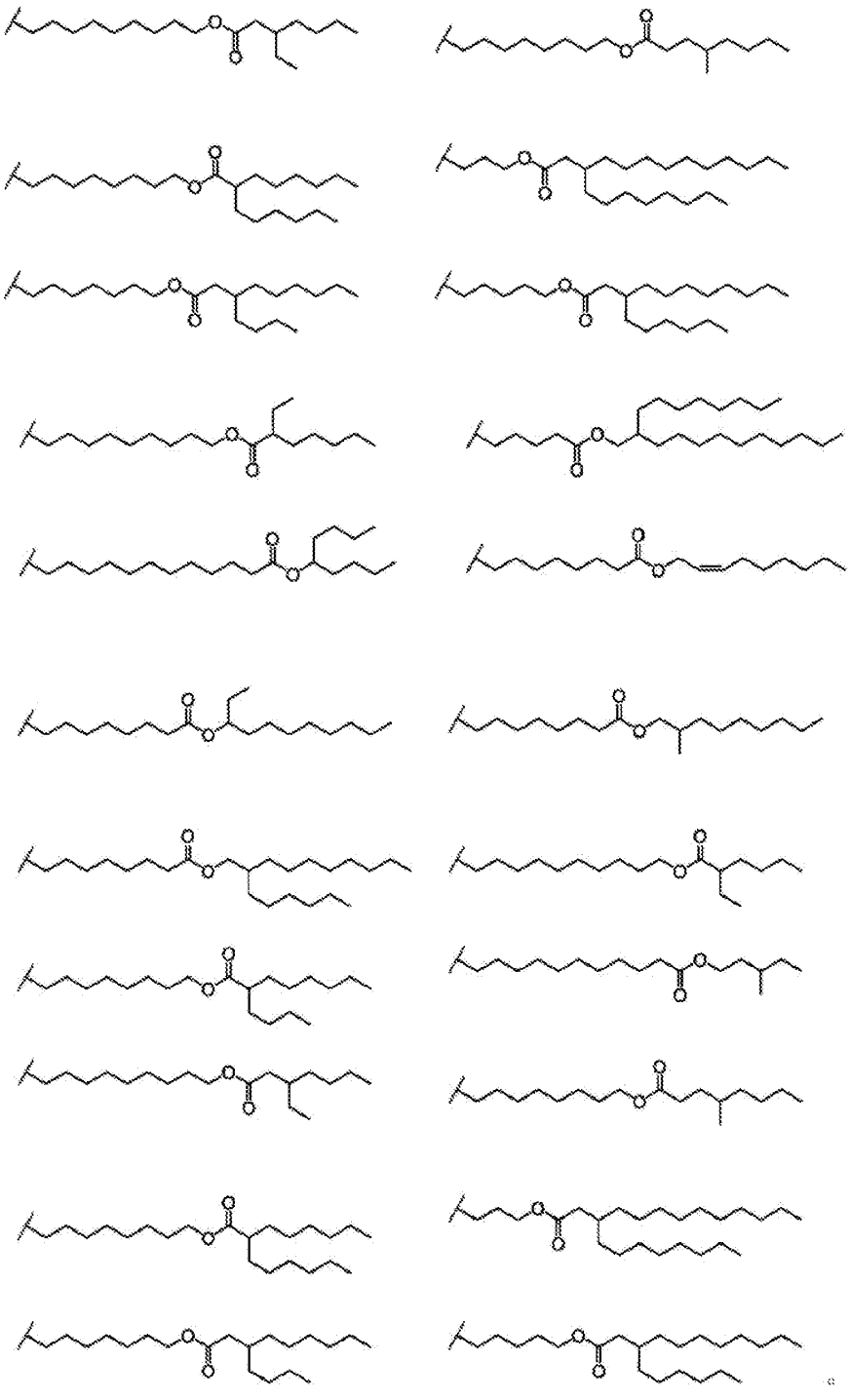
In some embodiments, X_2 is OC(O) or C(O)O; and -L_3-R" is independently selected from:



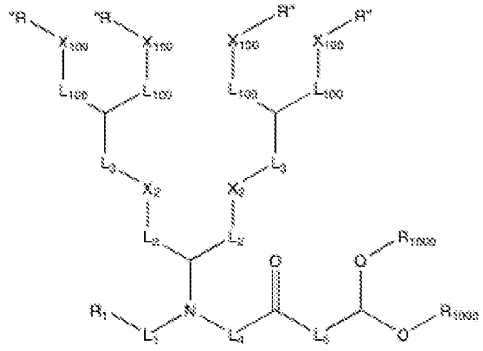








In some embodiments, a compound of formula (A-IV)



(A-IV): where each X_{100} is independently OC(O), C(O)O, OC(O)O, OC(O)NH, or NHC(O)O; L_{100} is C_1-C_6 akylene; and L_1-L_5, X_2, R_1, R" and R_{1000} are as previously defined.

In some embodiments, L_{100} is CH_2 and X_{100} is OC(O).

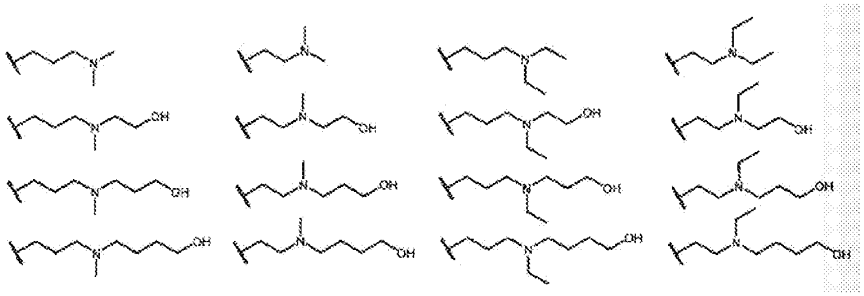
In some embodiments, L_4 is absent, and L_{100} is CH_2 and X_{100} is OC(O).

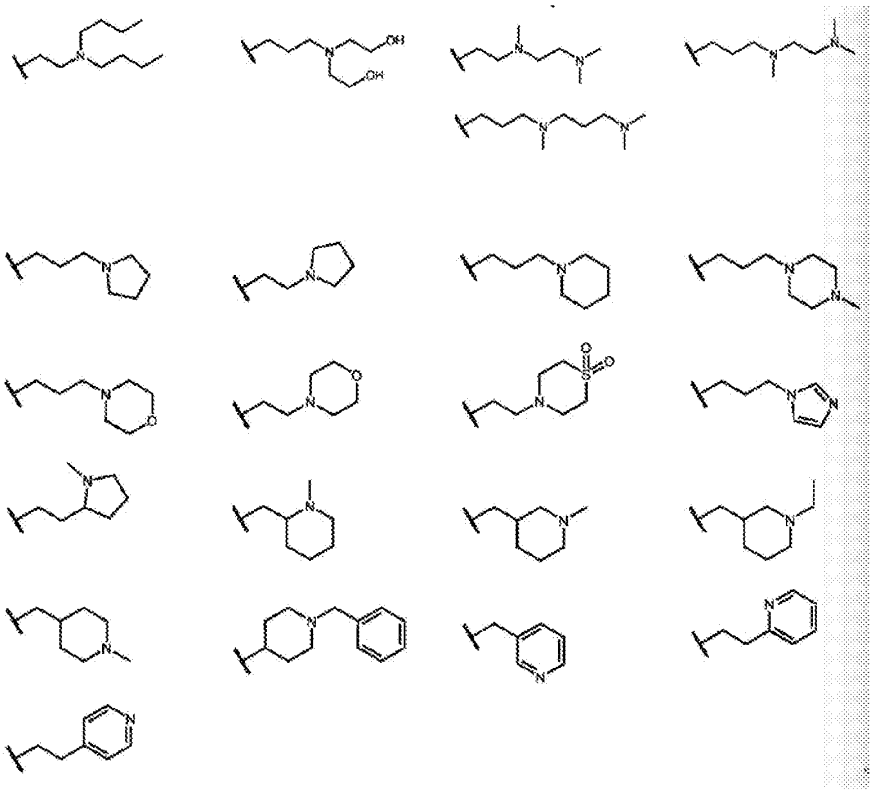
In some embodiments, L_2 is CH_2, X_2 is OC(O), and L_3 is CH_2.

In some embodiments, L_2 is CH_2, X_2 is OC(O), L_3 is CH_2, L_4 is absent, and L_{100} is CH_2 and X_{100} is OC(O).

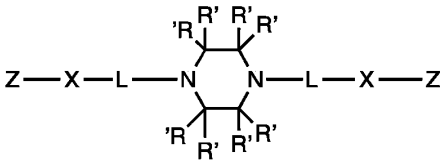
In some embodiments, L_1 is C2-C6 alkylene.

In some embodiments. -L_1-R_1 is selected from:





In some embodiments, a compound of formula (B-I):



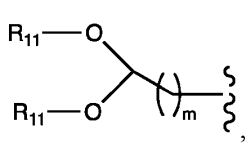
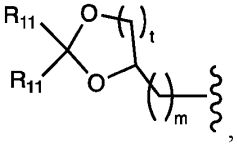
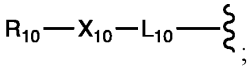
(B-I)

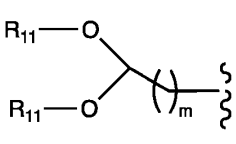
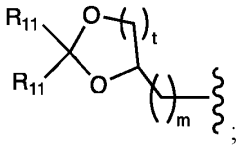
or its N-oxide, isomer, or a pharmaceutically acceptable salt thereof, wherein

each R' is independently absent, optionally substituted C_{1-6} alkyl, or optionally substituted C_{1-6} heteroalkyl;

each of L is independently optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

each of X is independently O, S, N(R"), S-S, OC(O), C(O)O, C(O)NH, NHC(O), OC(O)O, OC(O)NH, or NHC(O)O;

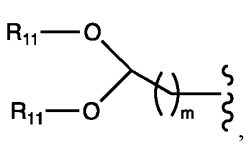
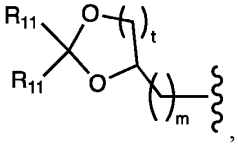
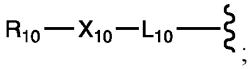
each Z is independently , , or ;

provided that at least one or two of Z is  or ;

each of m is independently 1-9;

t is 1, 2 or 3;

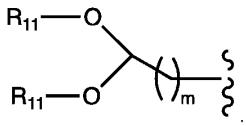
X_{10} is absent, O, S, N(R"), S-S, OC(O), C(O)O, C(O)NH, NHC(O), OC(O)O, OC(O)NH, or NHC(O)O;

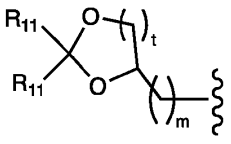
R" is H, , , or ;

L_{10} is optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

each of R_{11} is independently C_{2-20} aliphatic, 3- to 12-membered cycloaliphatic, C_2-C_{10} aliphatic substituted with 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, or phenyl; and

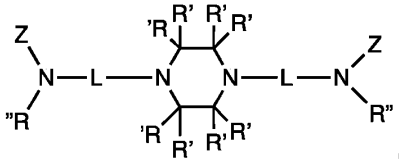
each of R_{10} is independently hydrogen, OR_6, or an optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, or phenyl.

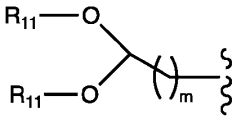
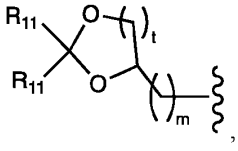
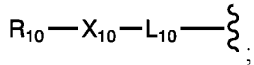
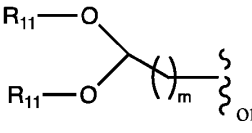
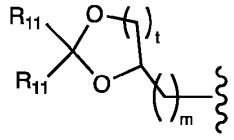
In some embodiments, Z is .

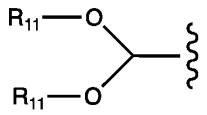
In some embodiments, Z is .

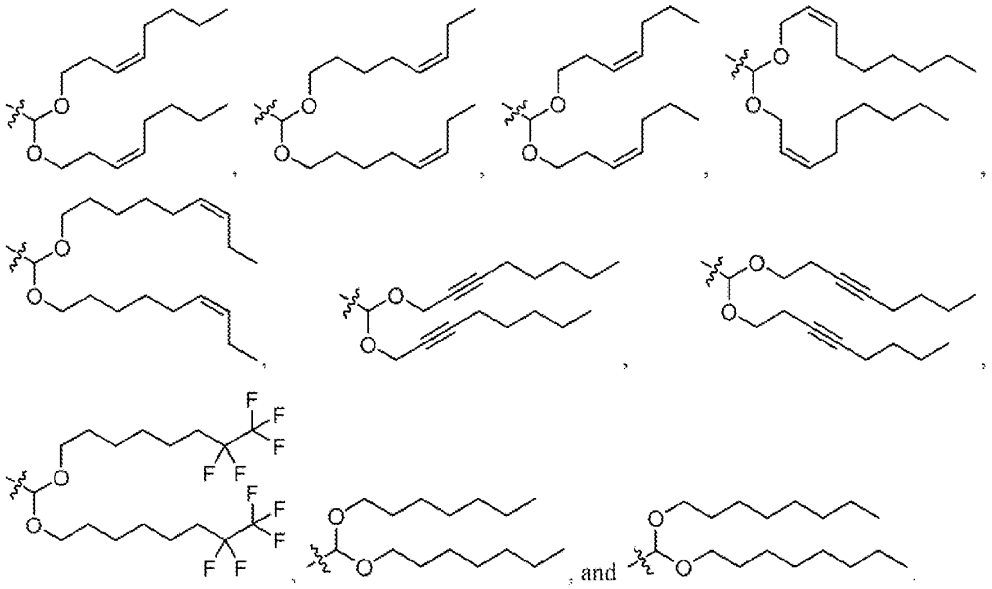
In some embodiments, R' is H or CH_3.

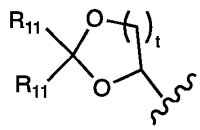
In some embodiments, a compound represented by formula (B-II):

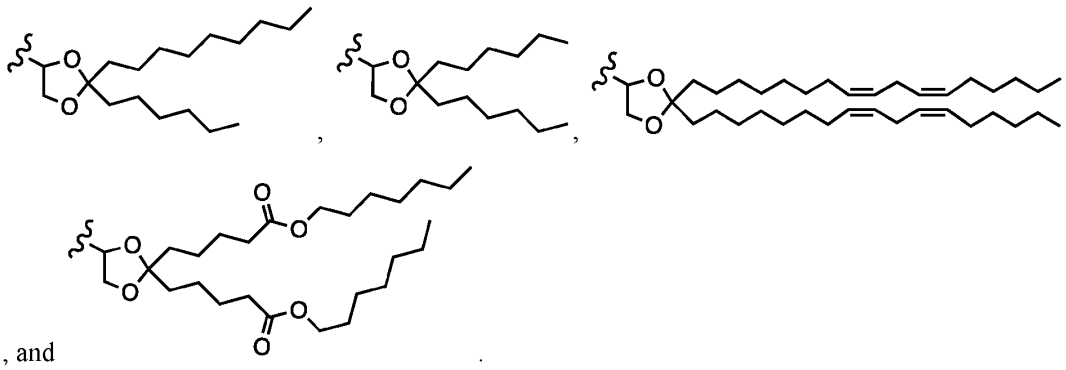

(B-II)

wherein Z and R" are each independently , , or ; provided at least two or three of Z and R" are  or .

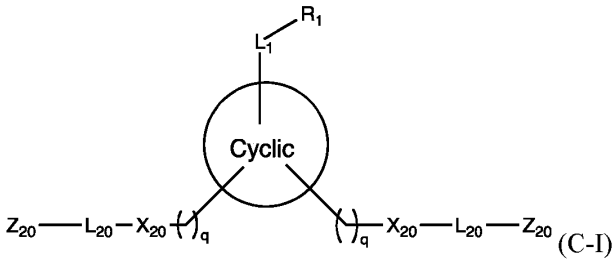
In some embodiments,  is selected from the group consisting of:



In some embodiments,  is selected from the group consisting of:



In some embodiments, a compound of formula (C-I):

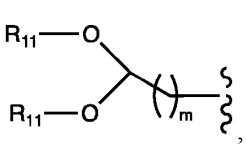
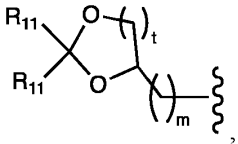
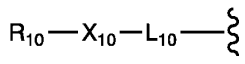
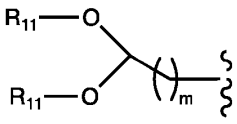
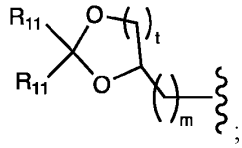


(C-I)

or its N-oxide, isomer, or a pharmaceutically acceptable salt thereof, wherein

each of X_{20} is independently absent, O, S, N(R"), S-S, OC(O), C(O)O, C(O)NH, NHC(O), OC(O)O, OC(O)NH, or NHC(O)O;

each of L_{20} is independently optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

each of Z_{20} is independently , , or ; provided that at least one of Z_{20} is  or ;

each of m is independently 1-9;

t is 1, 2 or 3;

L_{10} is optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

each of R_{11} is independently C_{2-20} aliphatic, 3- to 12-membered cycloaliphatic, C_2-C_{10} aliphatic substituted with 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, or phenyl; and

each of R_{10} is independently hydrogen, OR_6, or an optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, or phenyl;

q is 0, 1, 2, 3, 4, 5, 6, 7, 8, or 9.



is 4- to 8- membered cyclic or heterocyclic ring;

L_1 is absent, C_{1-6} alkylenyl, or C_{2-6} heteroalkylenyl;

R_1 is hydrogen, optionally substituted phenyl, optionally substituted 3- to 7-membered cycloaliphatic, optionally substituted 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 8- to 10-membered bicyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, $-OR_2$, $-C(O)OR_2$, $-C(O)SR_2$, $-OC(O)R_2$, $-OC(O)OR_2$, $-CN$, $-N(R_2)_2$, $-C(O)N(R_2)_2$, $-S(O)_2N(R_2)_2$, $-NR_2C(O)R_2$, $-OC(O)N(R_2)_2$, $-N(R_2)C(O)OR_2$, $-NR_2S(O)_2R_2$, $-NR_2C(O)N(R_2)_2$, $-NR_2C(S)N(R_2)_2$, $-NR_2C(NR_2)N(R_2)_2$, $-NR_2C(CHR_2)N(R_2)_2$, $-N(OR_2)C(O)R_2$, $-N(OR_2)S(O)_2R_2$, $-N(OR_2)C(O)OR_2$, $-N(OR_2)C(O)N(R_2)_2$, $-N(OR_2)C(S)N(R_2)_2$, $-N(OR_2)C(NR_2)N(R_2)_2$, $-N(OR_2)C(CHR_2)N(R_2)_2$, $-C(NR_2)N(R_2)_2$, $-C(NR_2)R_2$, $-C(O)N(R_2)OR_2$, $-C(R_2)N(R_2)_2C(O)OR_2$, $-CR_2(R_3)_2$, $-OP(O)(OR_2)_2$, or $-P(O)(OR_2)_2$; or

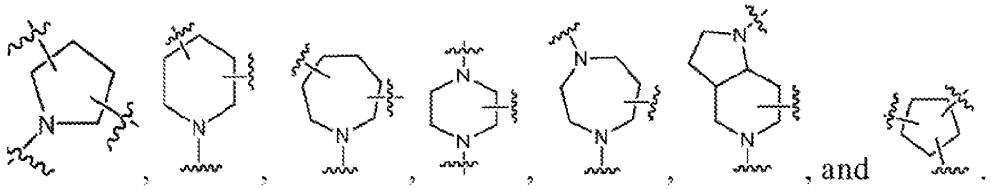
R_1 is a ring selected from 3- to 7-membered cycloaliphatic and 3- to 7- membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, wherein the cycloaliphatic or heterocyclyl ring is optionally substituted with 1-4 R_2 or R_3 groups;

each R_2 is independently hydrogen, oxo, -CN, $-NO_2$, $-OR_4$, $-S(O)_2R_4$, $-S(O)_2N(R_4)_2$, $-(CH_2)_n-R_4$, or an optionally substituted group selected from C_{1-6} aliphatic, phenyl, 3- to 7-membered cycloaliphatic, 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; or two occurrences of R_2, taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur; each R_3 is independently $-(CH_2)_n-R_4$; or two occurrences of R_3, taken together with the atom(s) to which they are attached, form optionally substituted 5- to 6-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur; each R_4 is independently hydrogen, $-OR_5$, $-N(R_5)_2$, $-OC(O)R_5$, $-OC(O)OR_5$, -CN, $-C(O)N(R_5)_2$, $-NR_5C(O)R_5$, $-OC(O)N(R_5)_2$, $-N(R_5)C(O)OR_5$, $-NR_5S(O)_2R_5$, $-NR_5C(O)N(R_5)_2$, $-NR_5C(S)N(R_5)_2$, or $-NR_5C(NR_5)N(R_5)_2$; and

each R_5 is independently hydrogen, or optionally substituted C_{1-6} aliphatic; or two occurrences of R_5, taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur; each R_6 is independently C_{4-12} aliphatic; and each n is independently 0 to 4.

In some embodiments, the 

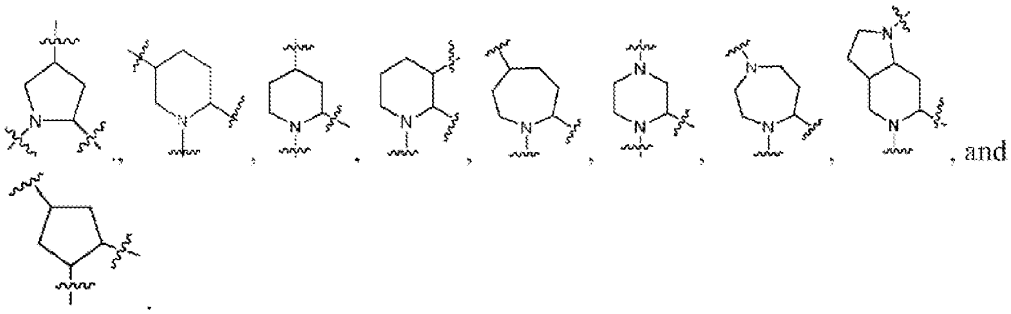
is selected from the group consisting of



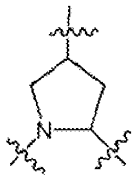
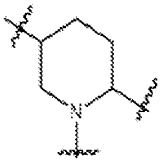
, and .

In some embodiments, the 

is selected from the group consisting of


, and
.

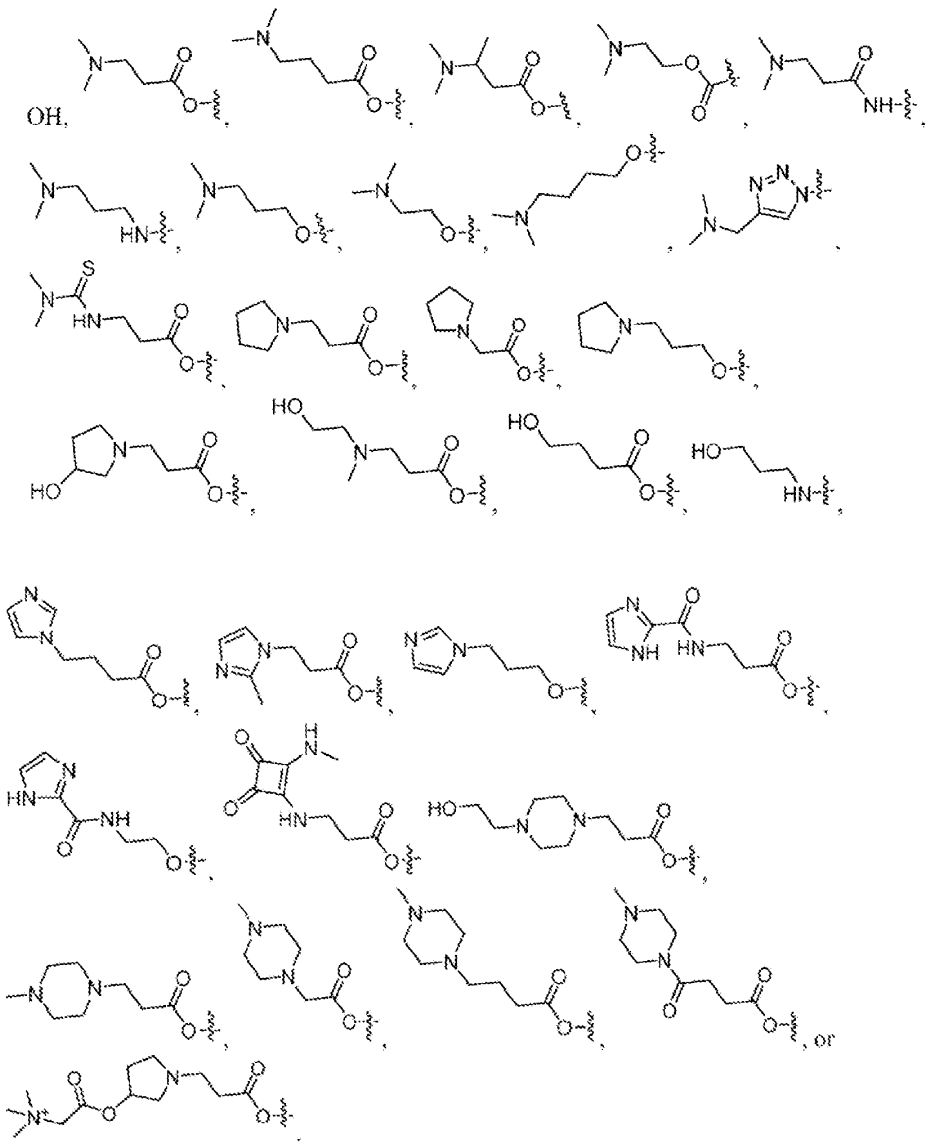
In some embodiments, the 

is  or .

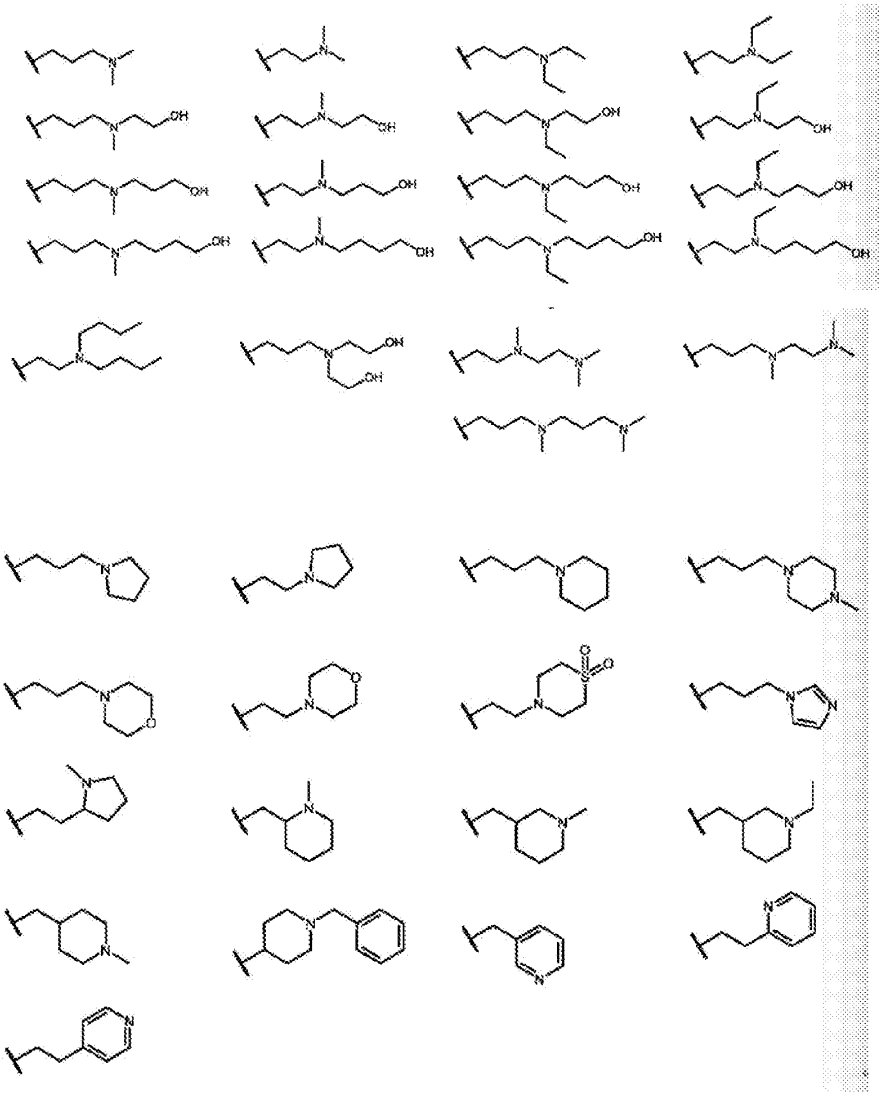
In some embodiments, L_1 is absent, and R_1 is $-OR_2$, $-C(O)OR_2$, $-C(O)SR_2$, $-OC(O)R_2$, $-OC(O)OR_2$, $-N(R_2)_2$, $-C(O)N(R_2)_2$, $-S(O)_2N(R_2)_2$, $-NR_2C(O)R_2$, $-OC(O)N(R_2)_2$, $-N(R_2)C(O)OR_2$, $-NR_2S(O)_2R_2$, or $-NR_2C(O)N(R_2)_2$; and R_2 is as defined above.

In some embodiments, R_1 is $-OR_2$ or $-C(O)OR_2$.

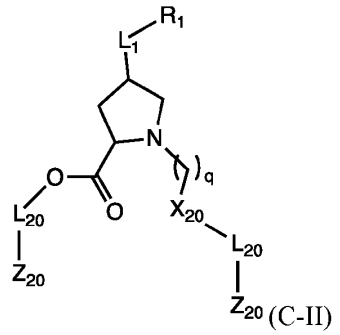
In some embodiments, L_1-R_1 is selected from the group consisting of



In some embodiments. -L_1-R_1 is selected from:



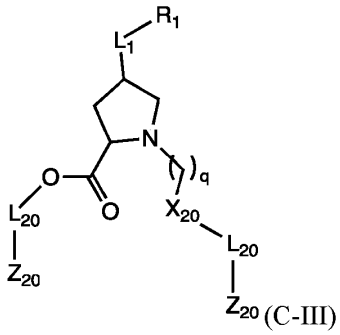
In some embodiments, the compound represented by formula (C-II):



(C-II)

wherein L_1 is absent, R_1 is OR_2, q is 2-9, X_{20} is OC(O) or C(O)O, R_2, L_{20} and Z_{20} are as defined above.

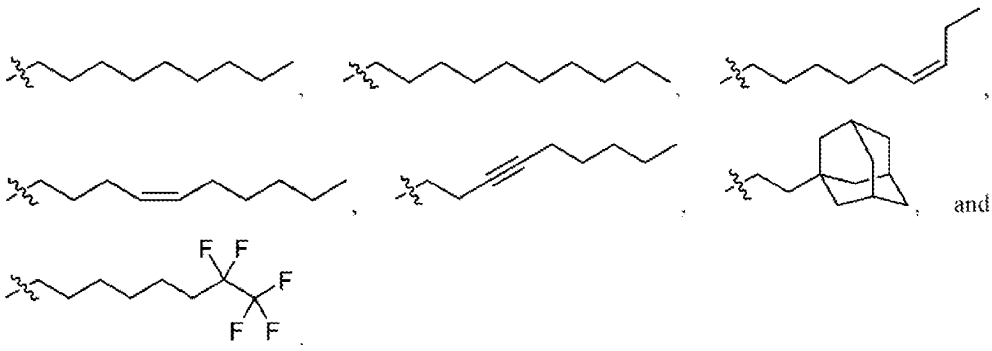
In some embodiments, a compound represented by formula (C-III):



(C-III)

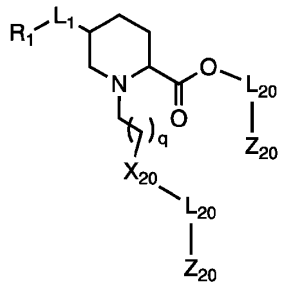
wherein L_1 is absent, R_1 is OR_2, q is 2-9, X_{20} is OC(O) or C(O)O, R_2, L_{20} and Z_{20} are as defined above.

In some embodiments, $-L_{20}-Z_{20}$ is selected from the group consisting of:



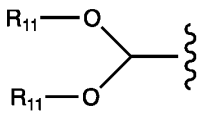
and

In some embodiments, a compound represented by formula (C-IV):

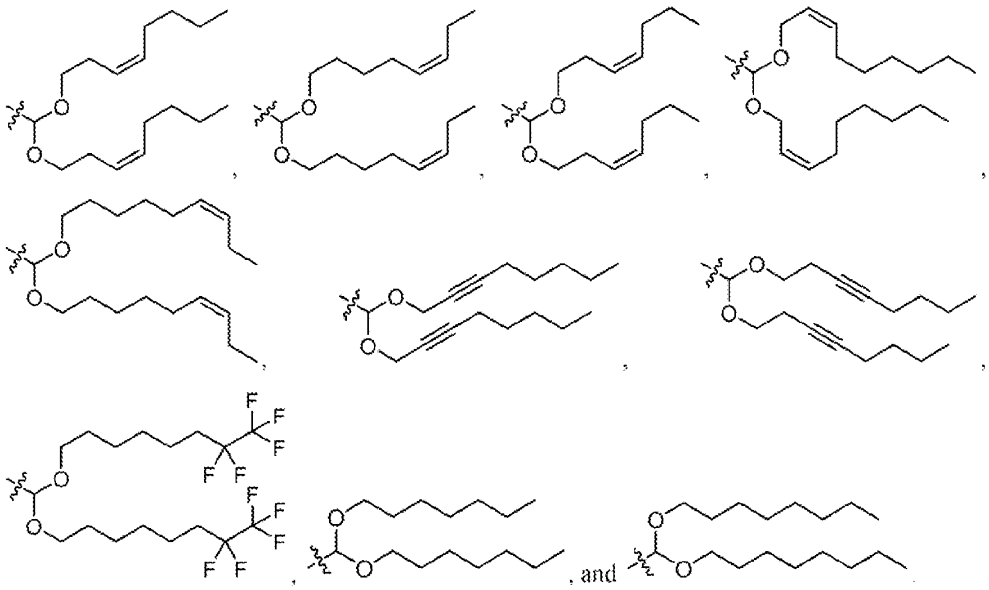


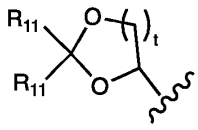
(C-IV)

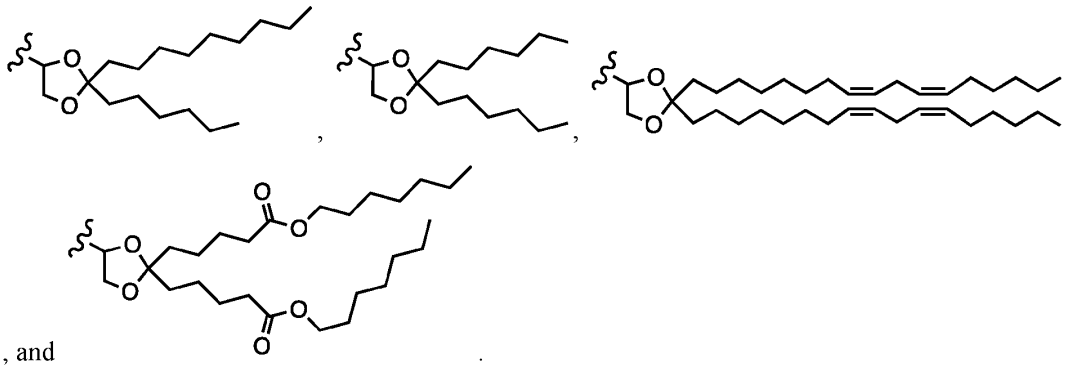
wherein L_1 is absent, R_1 is OR_2, q is 2-9, X_{20} is OC(O) or C(O)O, R_2, L_{20} and Z_{20} are as defined above.

In some embodiments, 

is selected from the group consisting of:

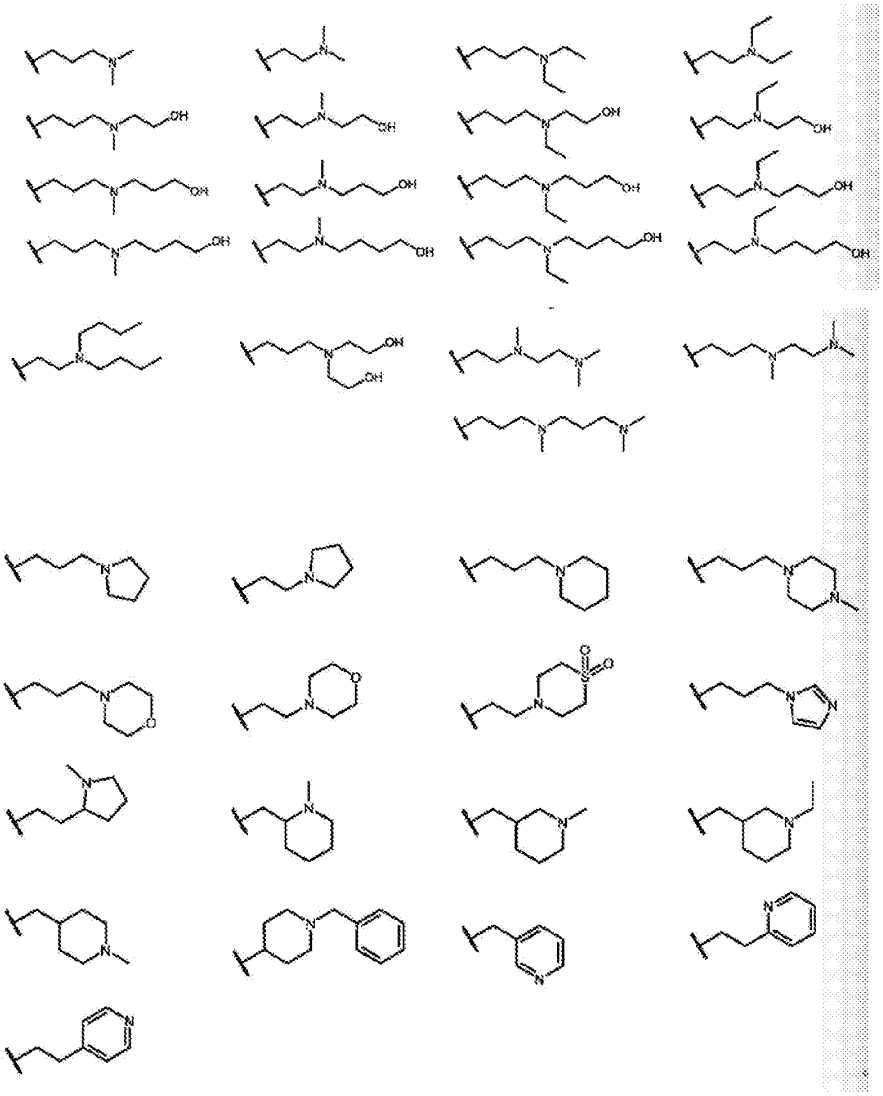


In some embodiments,  is selected from the group consisting of:

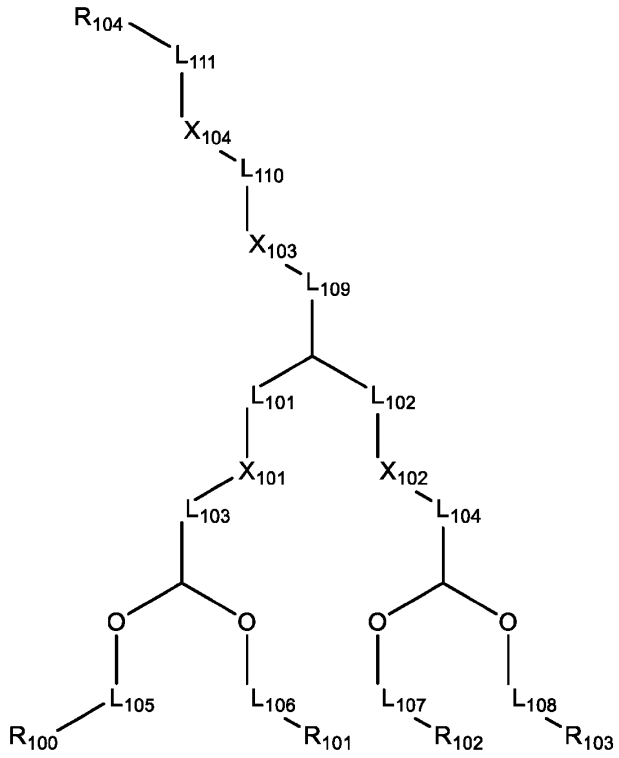


, and .

In some embodiments. -L_1-R_1 is selected from:



In some embodiments, a compound of formula (D-I):



or its N-oxide, isomer, or a pharmaceutically acceptable salt thereof, wherein

L_{101} and L_{102} are each independently C_{1-6} alkylenyl;

L_{103} and L_{104} are each independently optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

X_{101} and X_{102} are each independently absent, -OC(O)-, -C(O)O-, -NHC(O)-, -C(O)NH-, or -OC(O)O-;

L_{105}, L_{106}, L_{107} and L_{108} are each independently optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

R_{100}, R_{101}, R_{102}, and R_{103} are each independently hydrogen, OC_{1-3} aliphatic, OR_6, or an optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

L_{109} is C_{1-6} alkylenyl;

X_{103} is absent, -OC(O)-, -C(O)O-, -NHC(O)-, -C(O)NH-, -OC(O)O-, or -NR_{105}-;

R_{105} is hydrogen, C_{1-10} alkane, C_{2-10} alkene, or C_{3-12} cycloalkane;

L_{110} is C_{1-6} alkylenyl;

X_{104} is absent or -NR_{106}-;

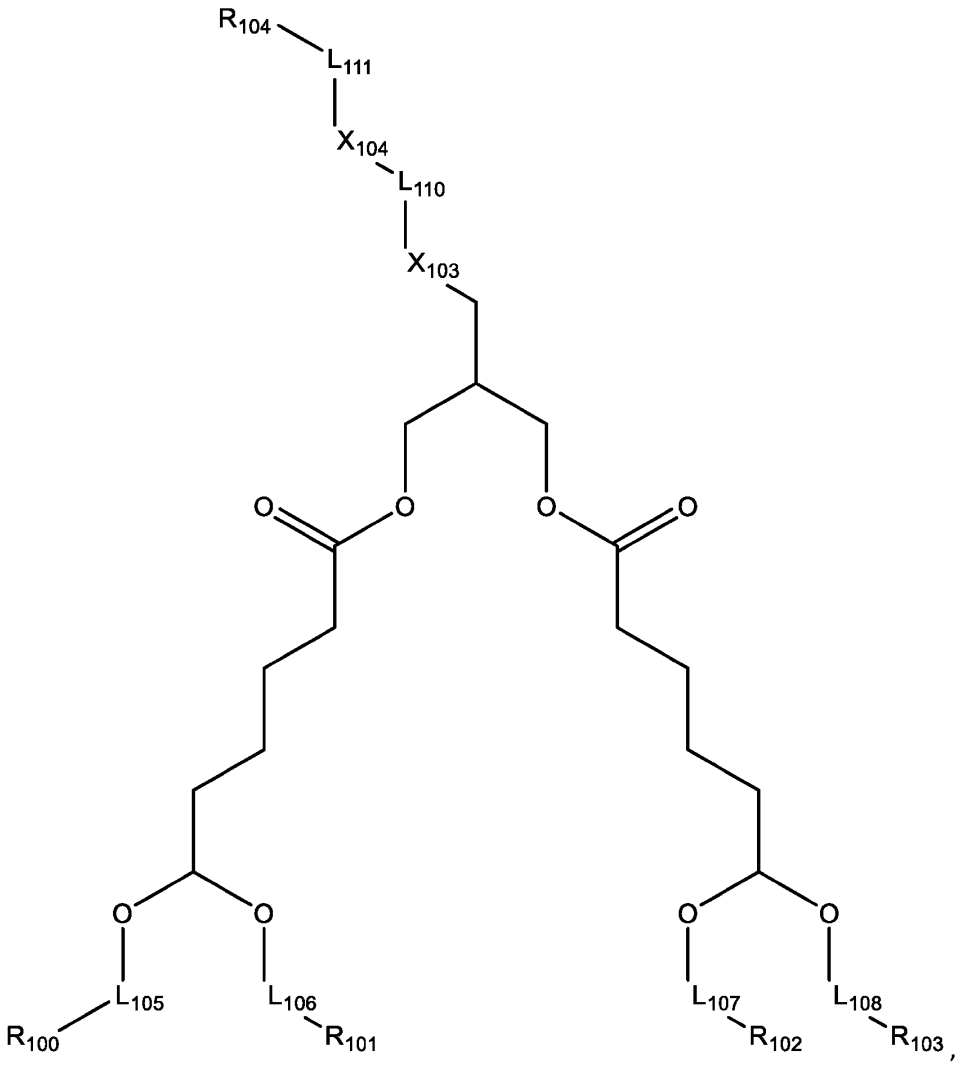
L_{111} is C_{1-6} alkylenyl;

R_{106} is hydrogen, C_{1-10} alkane, C_{2-10} alkene, or C_{3-12} cycloalkane;

R_{104} is hydrogen, OR_{107}, C_{1-10} alkane, C_{2-10} alkene, C_{3-12} cycloalkane, or an optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl; and

R_{107} is H or C_{1-12} aliphatic.

In some embodiments, a compound of formula (D-II):



wherein R_{100}, R_{101}, R_{102}, and R_{103} are each independently hydrogen, OC_{1-3} aliphatic, OR_6, or an optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, or phenyl;

L_{105}, L_{106}, L_{107} and L_{108} are each independently optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

X_{103} is absent, -OC(O)-, -C(O)O-, -NHC(O)-, -C(O)NH-, -OC(O)O-, or $-NR_{105}$-;

R_{105} is hydrogen, C_{1-10} alkane, C_{2-10} alkene, or C_{3-12} cycloalkane;

L_{110} is C_{1-6} alkylenyl;

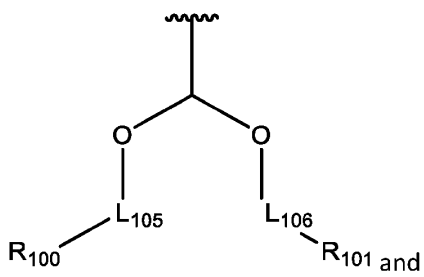
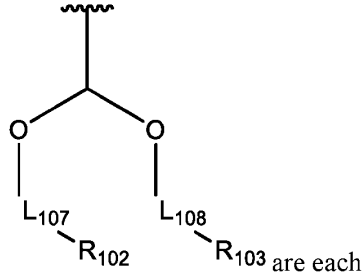
X_{104} is absent or -NR_{106}-;

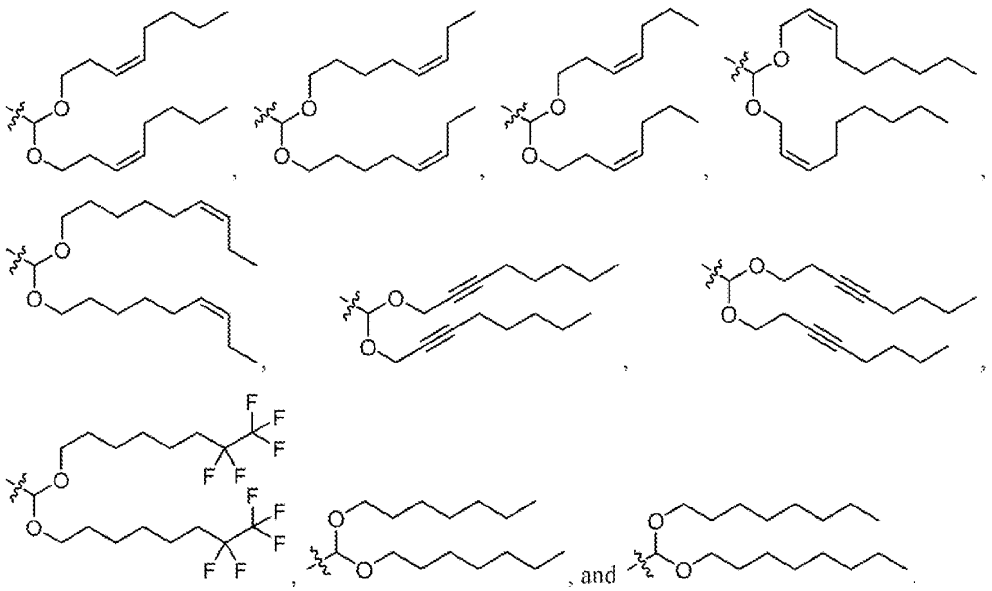
L_{111} is C_{1-6} alkylenyl;

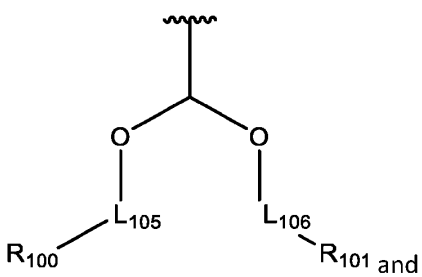
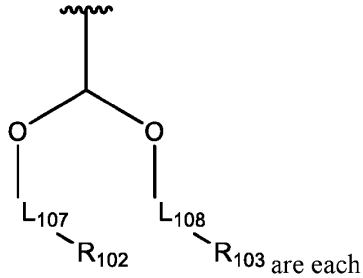
R_{106} is hydrogen, C_{1-10} alkane, C_{2-10} alkene, or C_{3-12} cycloalkane; and

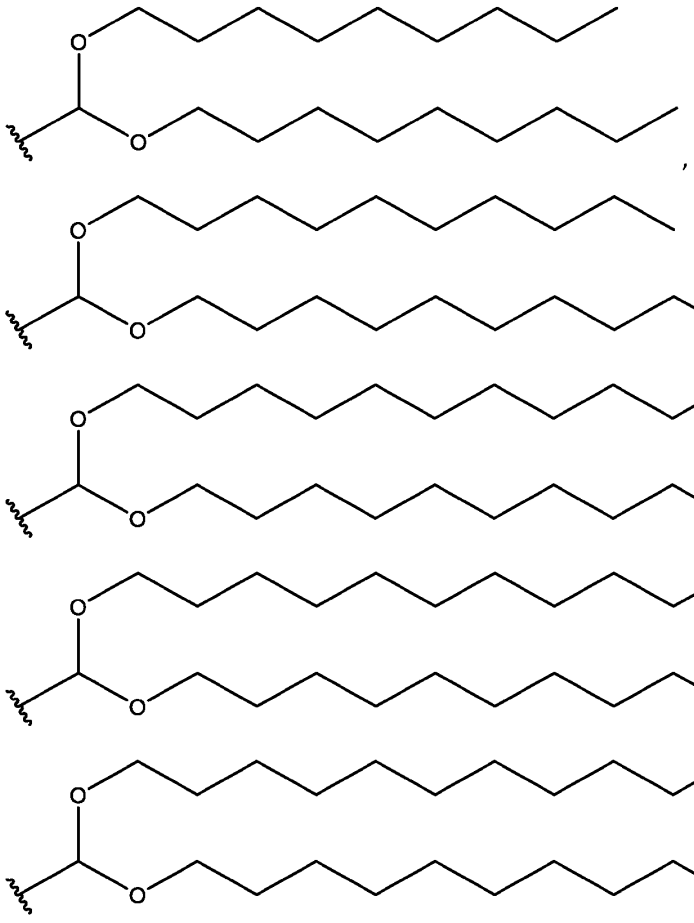
R_{104} is hydrogen, OR_{107}, C_{1-10} alkane, C_{2-10} alkene, C_{3-12} cycloalkane, or an optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, or phenyl; and

R_{107} is H or C_{1-12} aliphatic.

In some embodiments,  and  are each independently selected from the group consisting of:

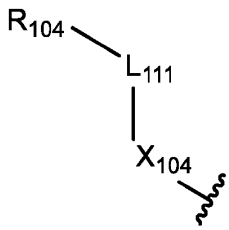


In some embodiments,  and  are each independently selected from the group consisting of:

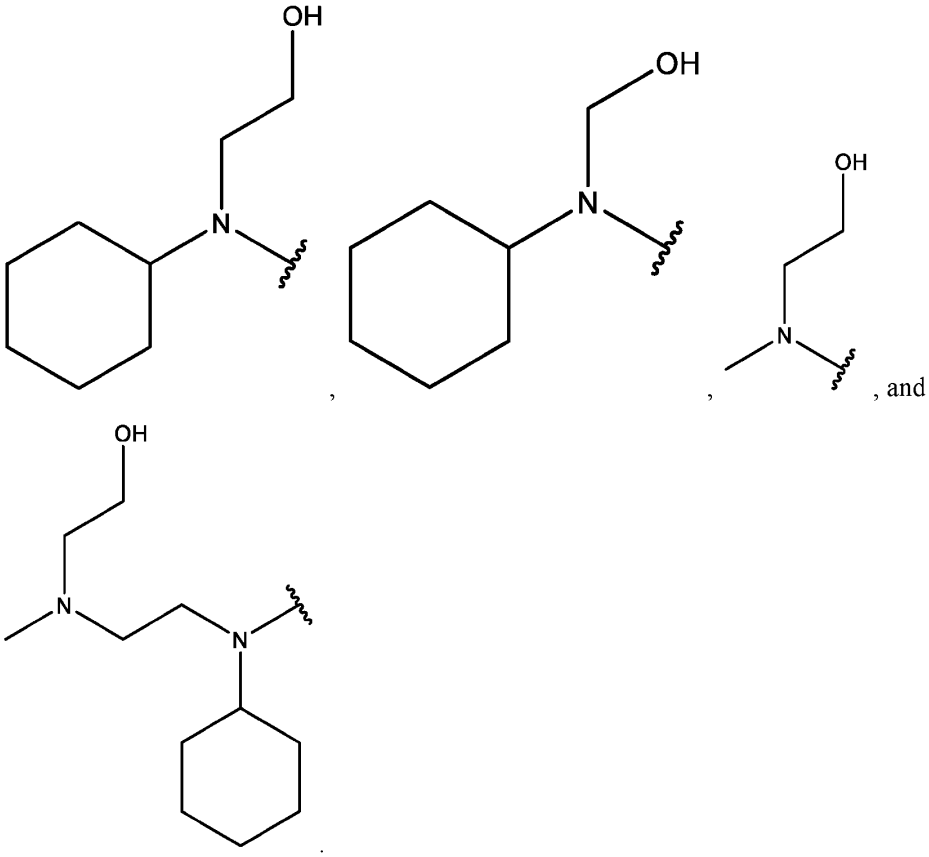


, and

.

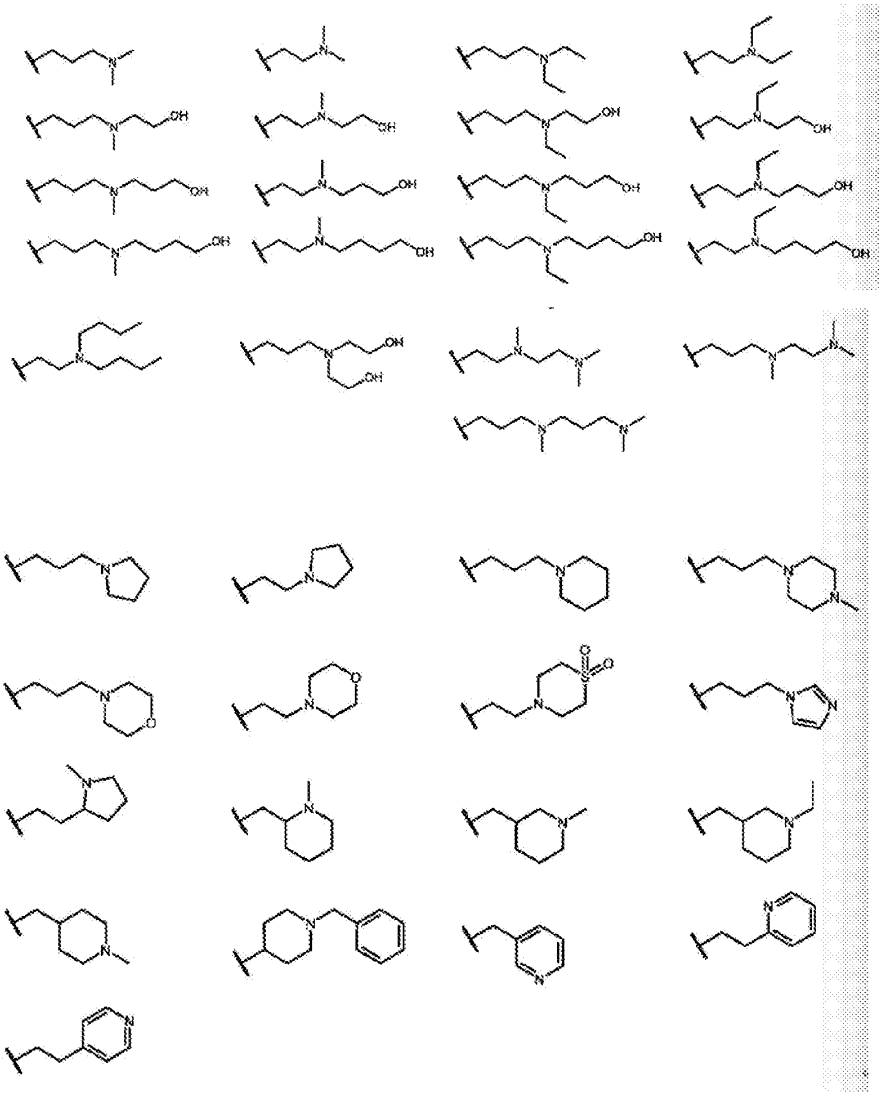


In some embodiments, is selected from the group consisting of:

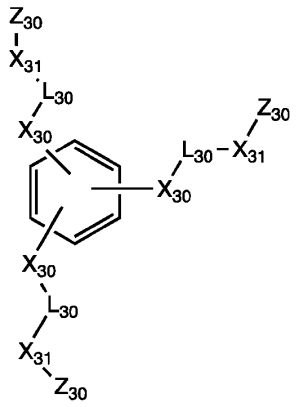


, , , and .

In some embodiments. -L_1-R_1 is selected from:



In some embodiments, a compound of formula (E-I):

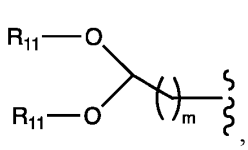
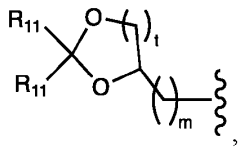
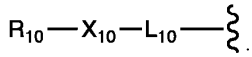
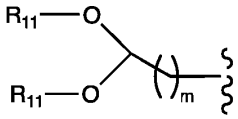
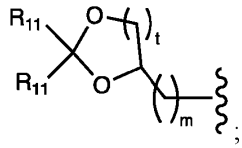


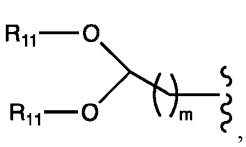
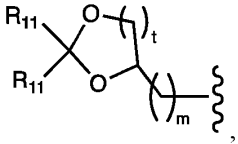
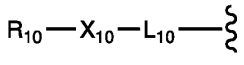
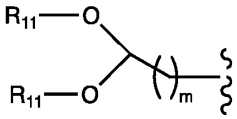
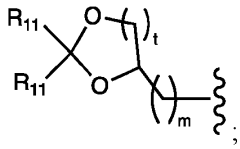
(E-I)

or its N-oxide, isomer, or a pharmaceutically acceptable salt thereof, wherein

each of X_{30} is independently absent, O, S, N(R"), S-S, OC(O), C(O)O, C(O)NH, NHC(O), OC(O)O, OC(O)NH, or NHC(O)O;

each of L_{30} is independently optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

each of X_{31} is independently NR"; wherein R" is H, , , or ; provided that at least one of Z_{30} is  or ;

each of Z_{30} is independently , , or ; provided that at least one of Z_{30} is  or ;

each of m is independently 1 to 9;

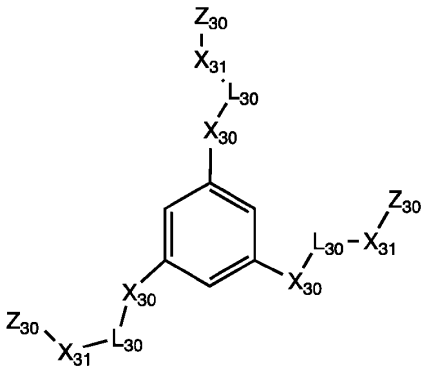
t is 1, 2 or 3;

L_{10} is optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

each of R_{11} is independently C_{2-20} aliphatic, 3- to 12-membered cycloaliphatic, C_2-C_{10} aliphatic substituted with 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, or phenyl; and

each of R_{10} is an optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, or phenyl.

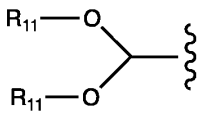
In some embodiments, a compound represented by formula (E-II)

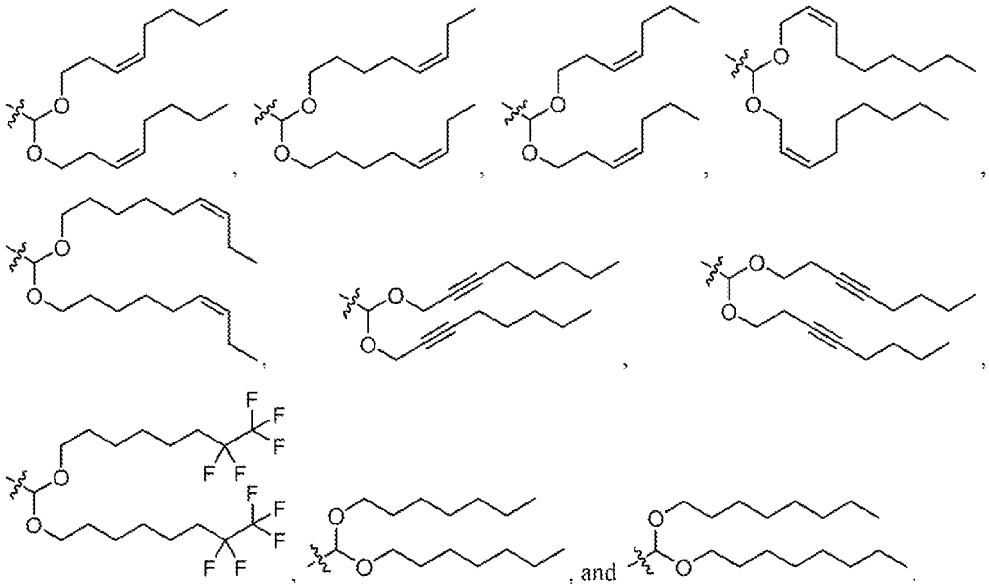


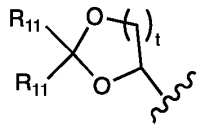
(E-II)

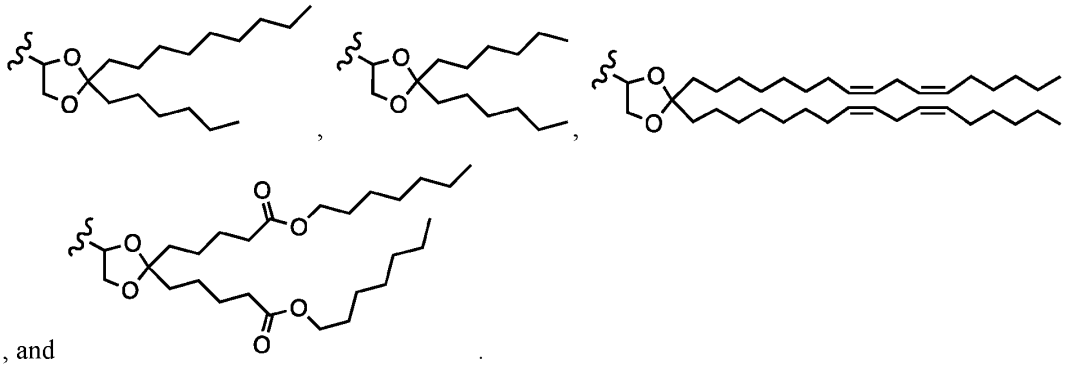
wherein X_{30}, L_{30}, X_{31} and Z_{30} are as defined above.

In some embodiments, X_{30} is C(O)O, OC(O), C(O)NH, NHC(O) or O.

In some embodiments,  is selected from the group consisting of:



In some embodiments,  is selected from the group consisting of:



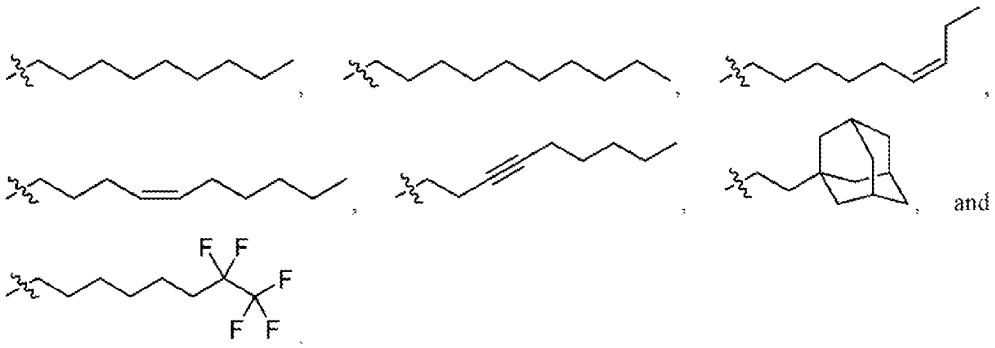
, and .

In some embodiments, X_{30} is C(O)NH.

In some embodiments, X_{30} is C(O)O.

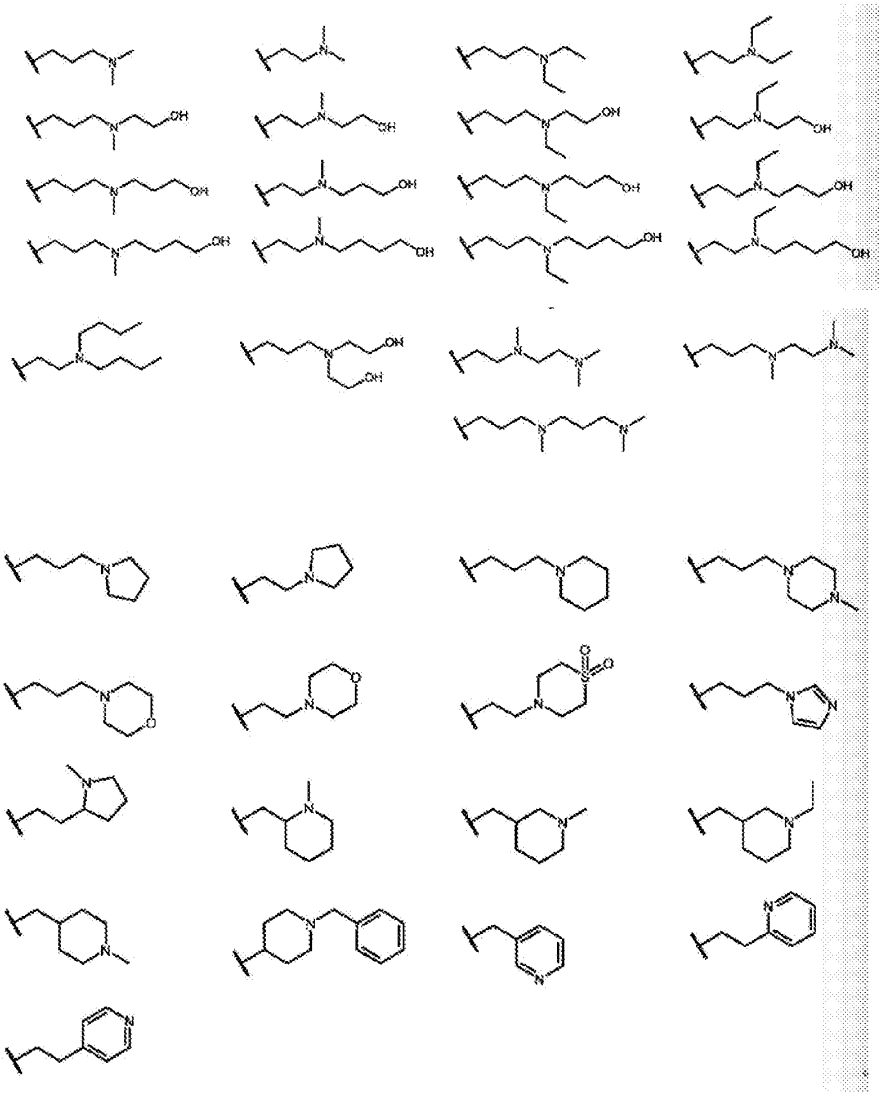
In some embodiments, X_{31} is N-R".

In some embodiments, Z_{30} is selected from the group consisting of:



and

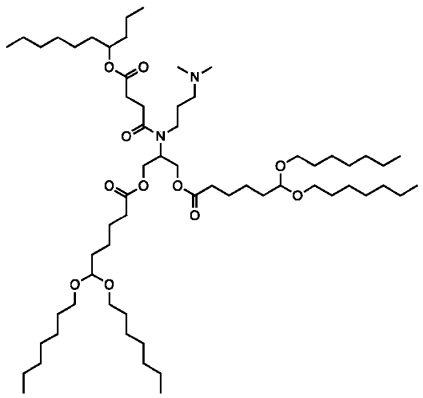
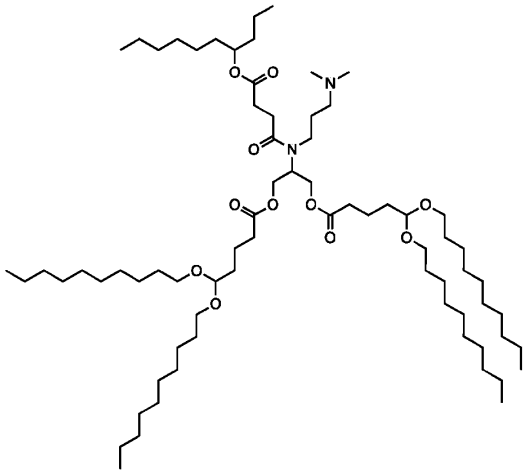
In some embodiments. $-L_1-R_1$ is selected from:

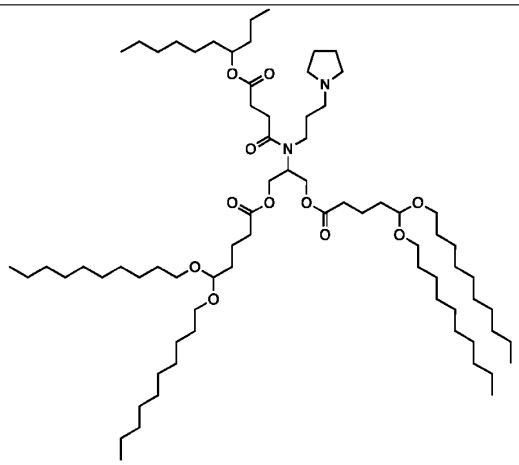
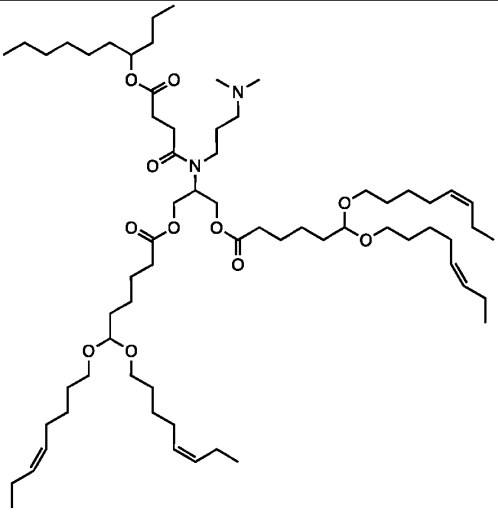


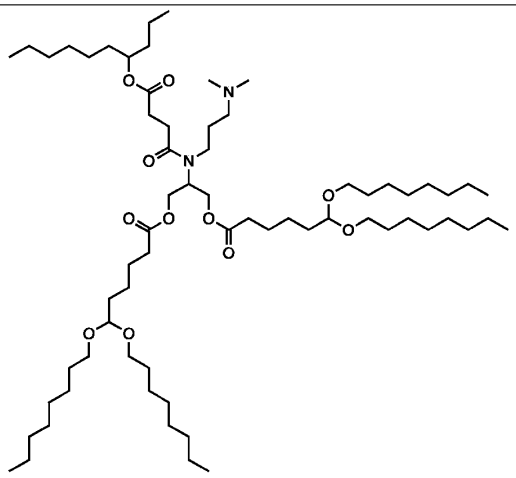
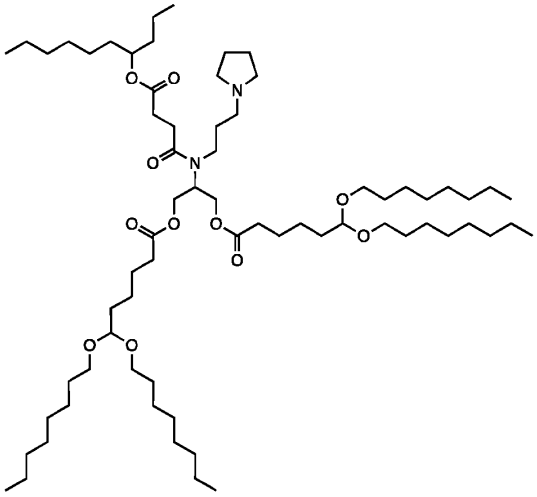
In some embodiments, the pKa of the protonated form of the compound is from about 4.5 to about 8.0.

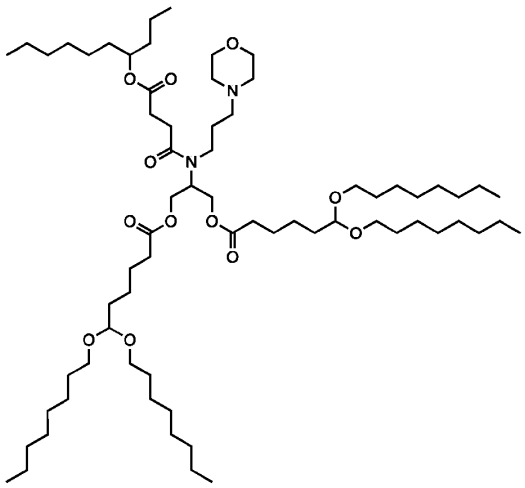
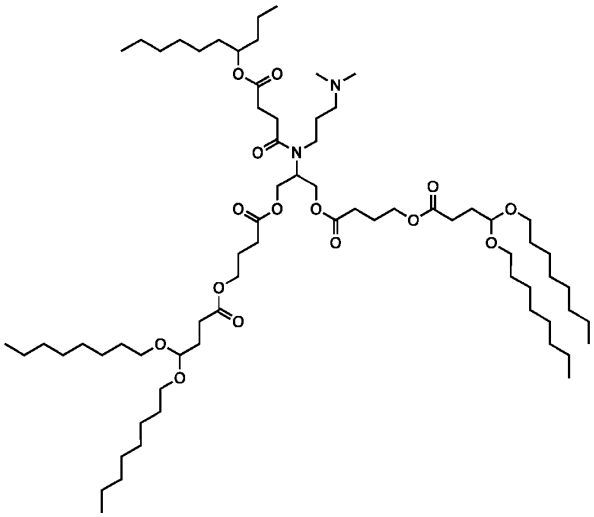
In some embodiments, the cationic lipid can be selected from an ionizable cationic lipid set forth in tables below, or an isomer or a salt thereof.

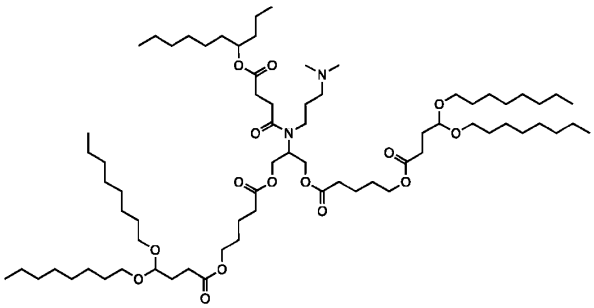
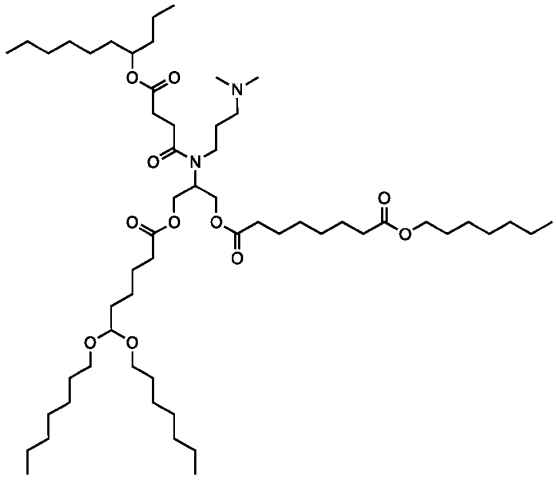
Table A

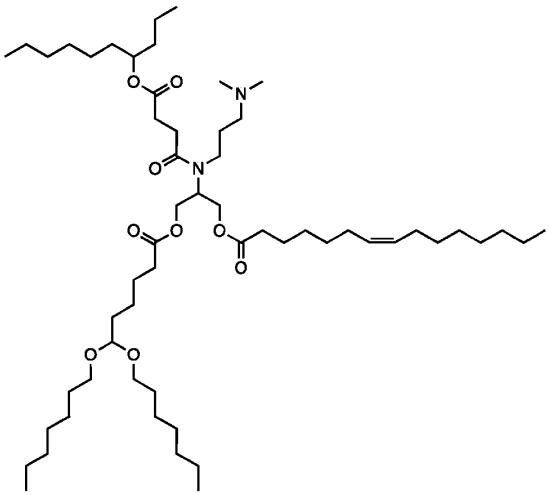
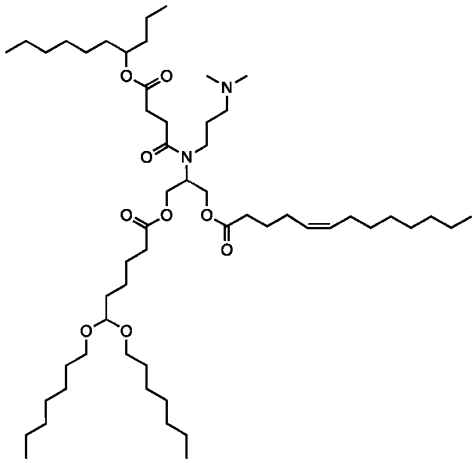
Compound	Structure	Name
A-1		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(heptyloxy)hexanoate)
A-2		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

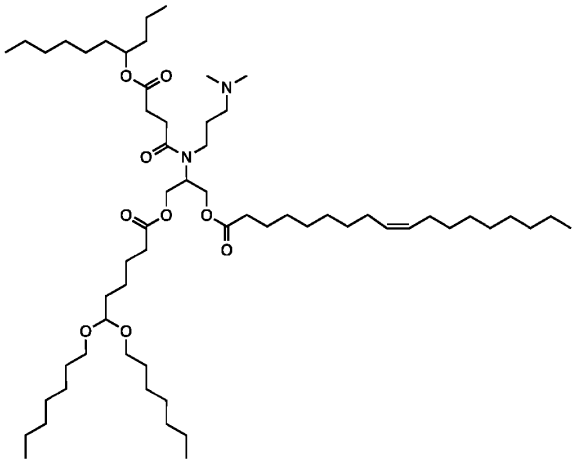
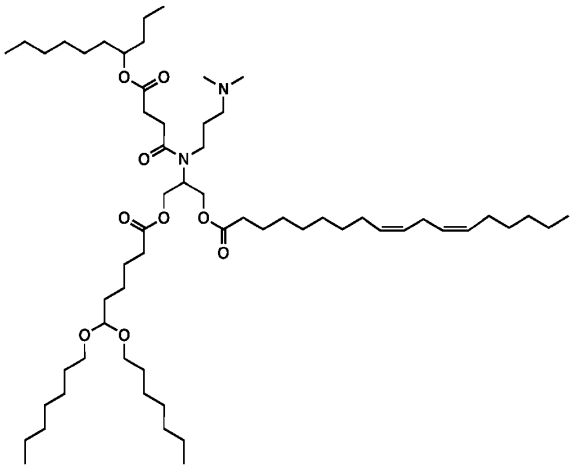
A-3		2-(4-(decan-4-yloxy)-4-oxo-N-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-4		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)

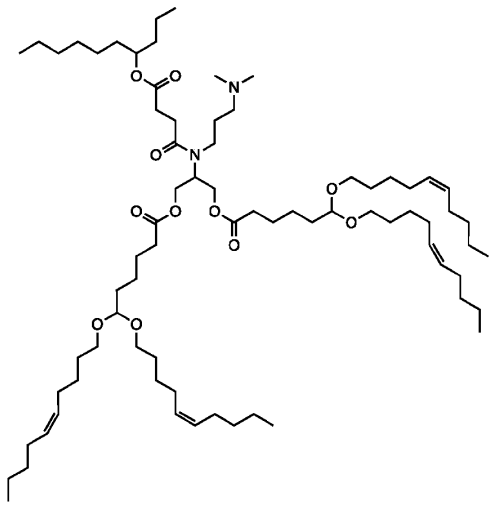
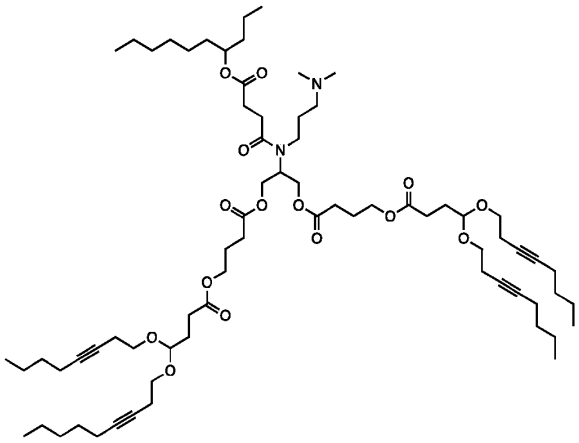
A-5		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-6		2-(4-(decan-4-yloxy)-4-oxo-N-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)

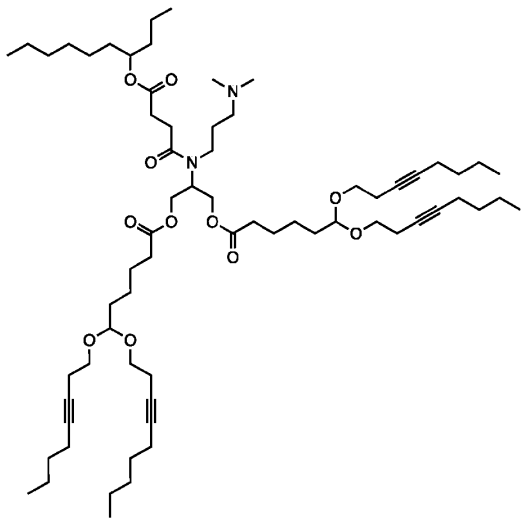
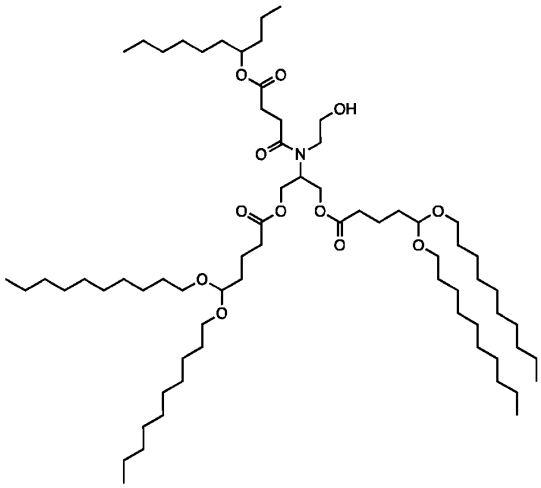
A-7		2-(4-(decan-4-yloxy)-N-(3-morpholinopropyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-8		((2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(4,4-bis(octyloxy)butanoate)

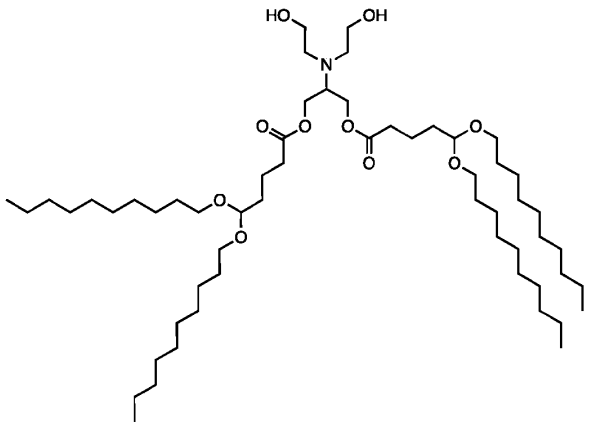
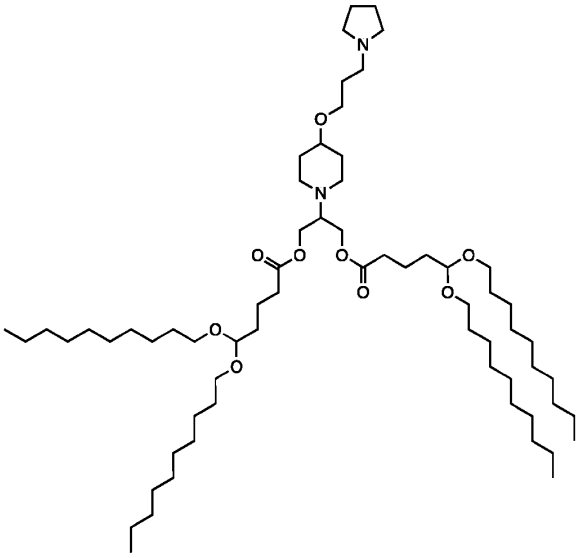
A-9		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(5-((4,4-bis(octyloxy)butanoyl)oxy)pentanoate)
A-10		1-(3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl) 8-heptyl octanedioate

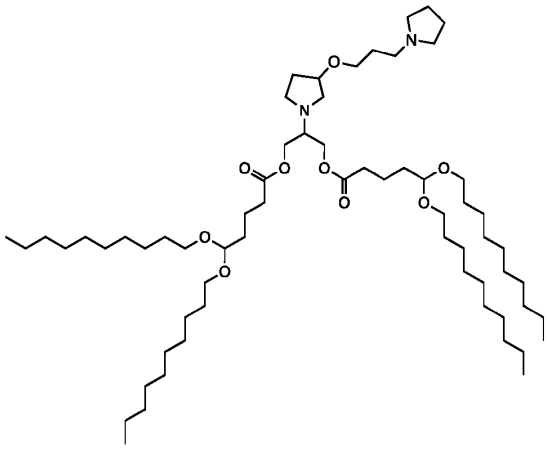
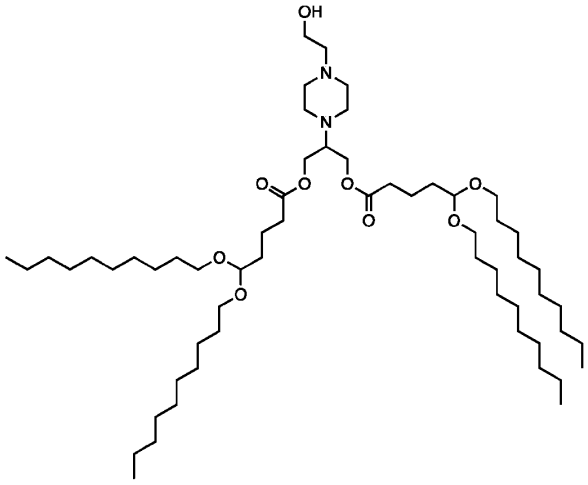
A-11		3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl (Z)-pentadec-7-enoate
A-12		3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl (Z)-tridec-5-enoate

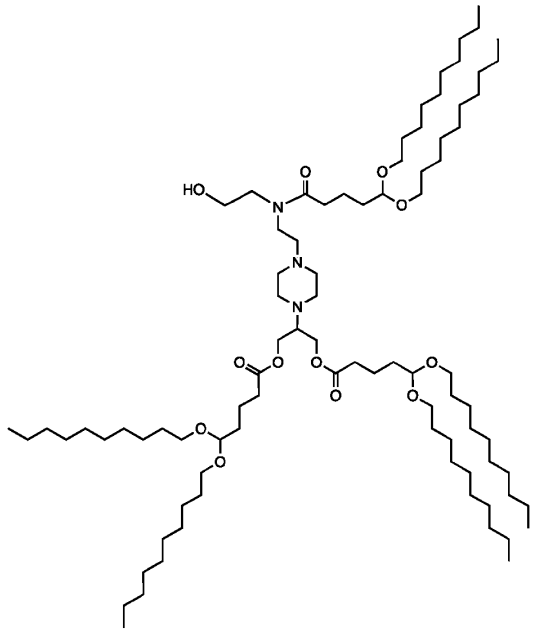
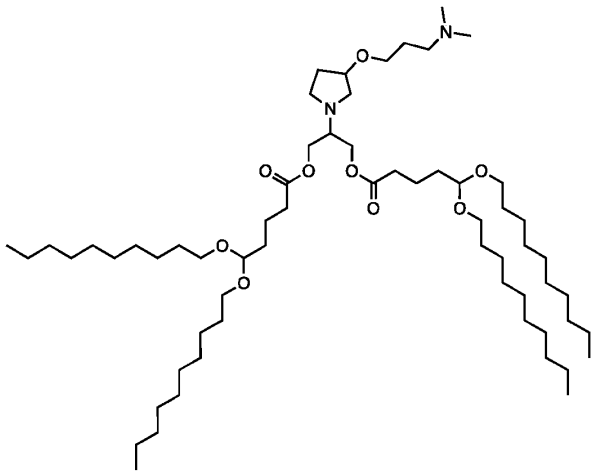
A-13		3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl oleate
A-14		3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl (9Z,12Z)-octadeca-9,12-dienoate

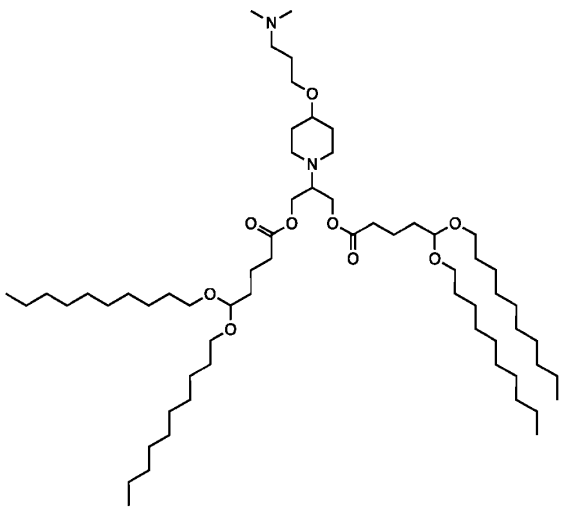
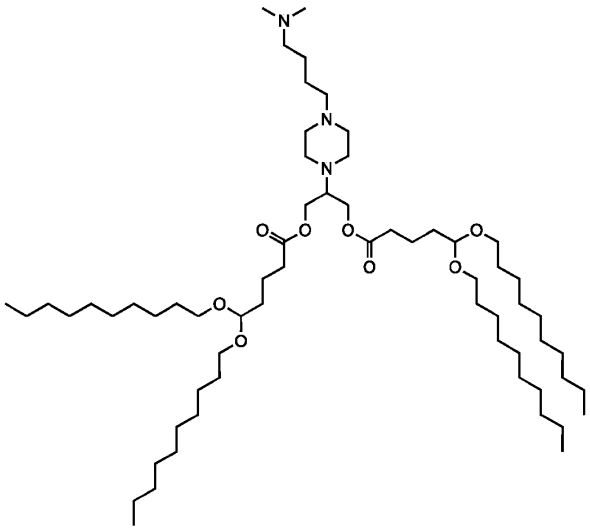
A-15		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((Z)-dec-5-en-1-yl)oxy)hexanoate)
A-16		decan-4-yl 4-((10,32-bis(oct-3-yn-1-yloxy)-13,18,24,29-tetraoxo-9,14,19,23,28,33-hexaoxadotetraconta-5,36-diyn-21-yl)(3-(dimethylamino)propyl)amino)-4-oxobutanoate

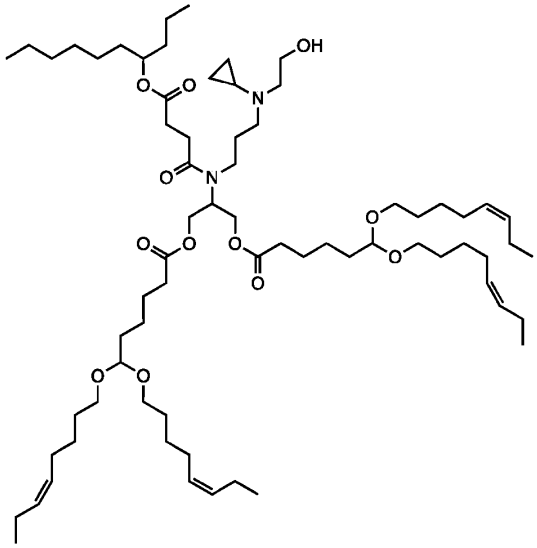
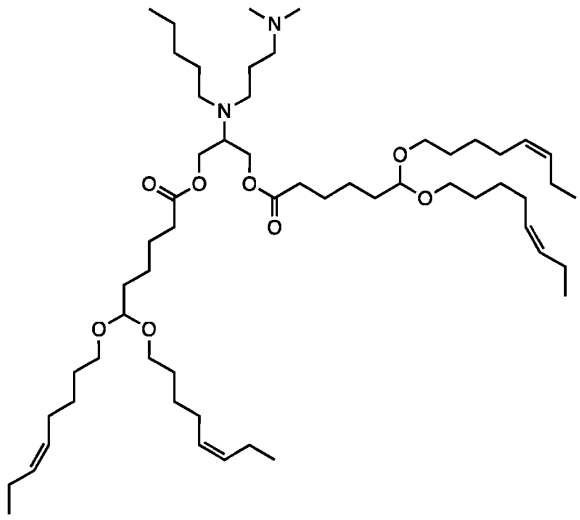
A-17		3-((6,6-bis(oct-3-yn-1-yloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl 6-(non-3-yn-1-yloxy)-6-(oct-3-yn-1-yloxy)hexanoate
A-18		2-(4-(decan-4-yloxy)-N-(2-hydroxyethyl)-4-oxobutanamido)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

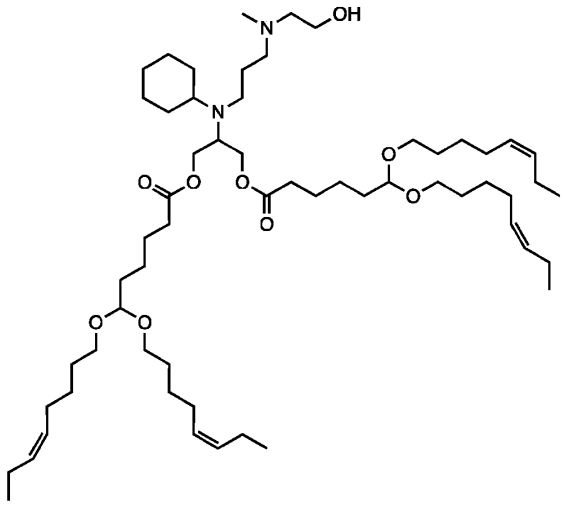
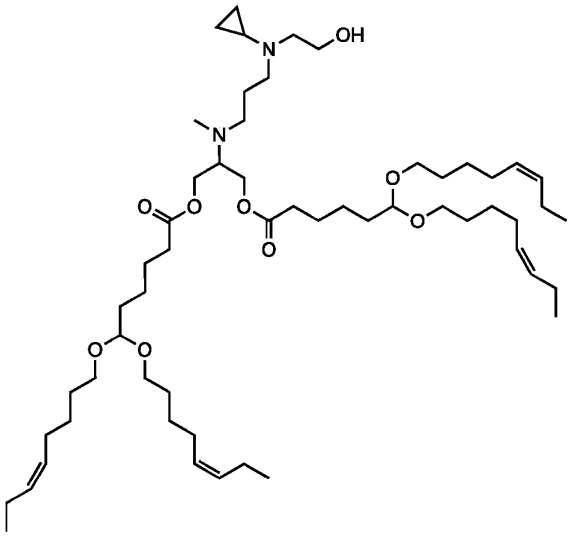
A-19		2-(bis(2-hydroxyethyl)amino)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-20		2-(4-(3-(pyrrolidin-1-yl)propoxy)piperidin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

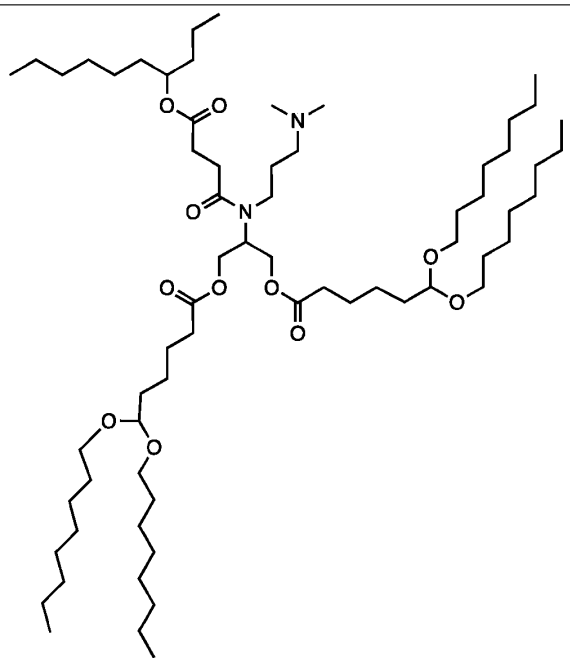
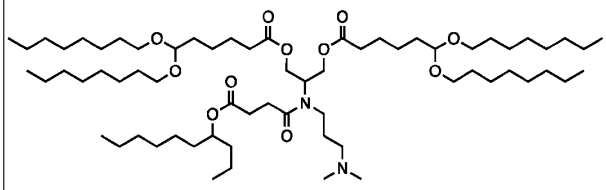
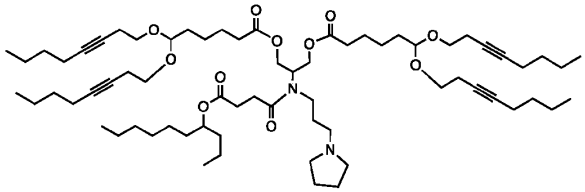
A-21		2-(3-(3-(pyrrolidin-1-yl)propoxy)pyrrolidin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-22		2-(4-(2-hydroxyethyl)piperazin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

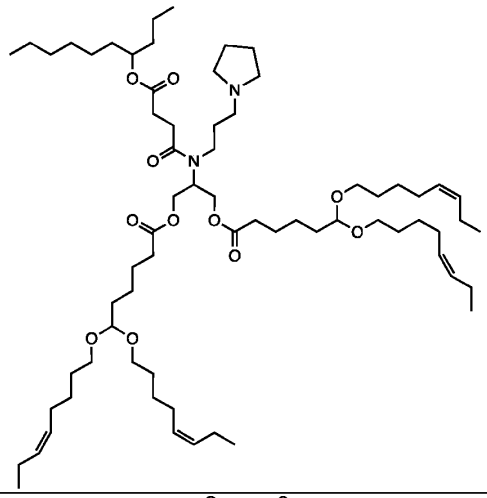
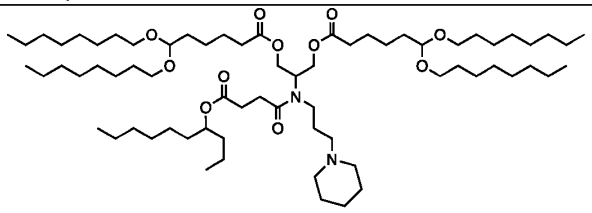
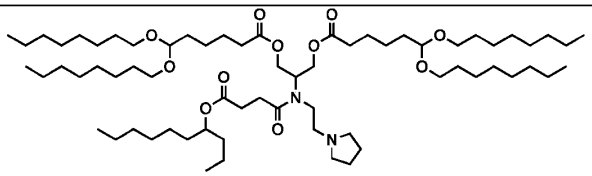
A-23		2-(4-(2-(5,5-bis(decyloxy)-N-(2-hydroxyethyl)pentanamido)ethyl)piperazin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-24-1		2-(3-(3-(dimethylamino)propoxy)pyrrolidin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

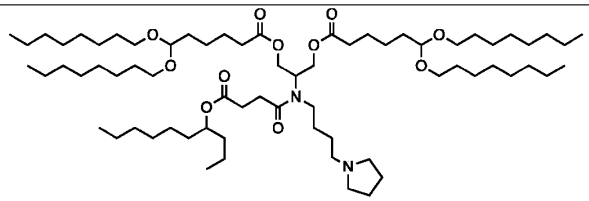
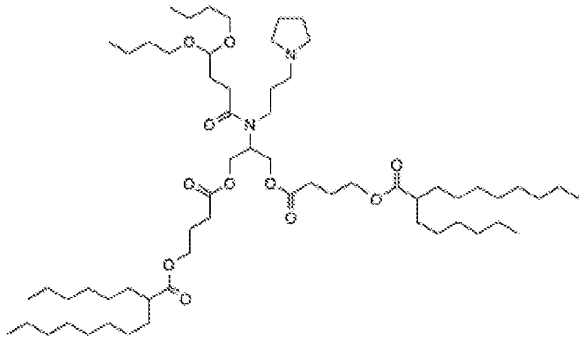
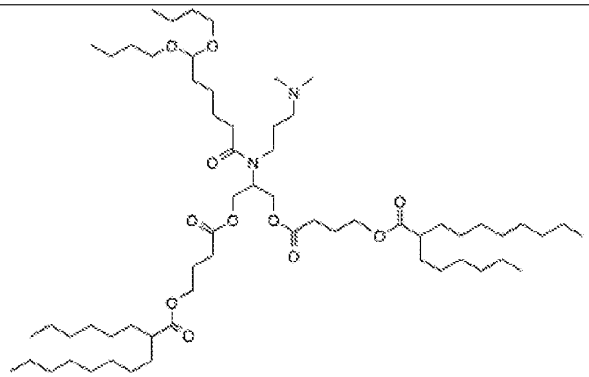
A-24		2-(4-(3-(dimethylamino)propoxy)piperidin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-25		2-(4-(4-(dimethylamino)butyl)piperazin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

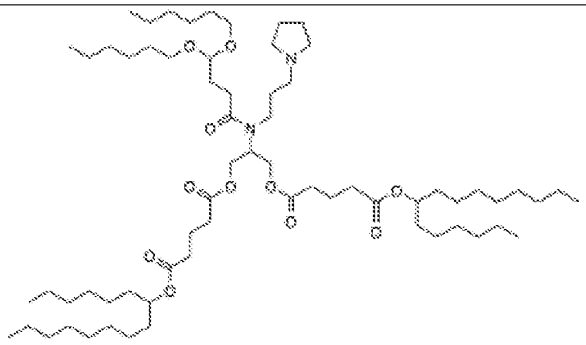
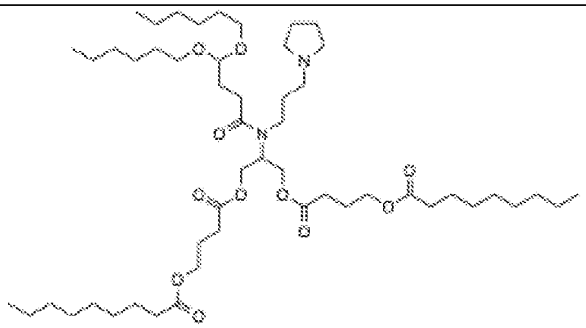
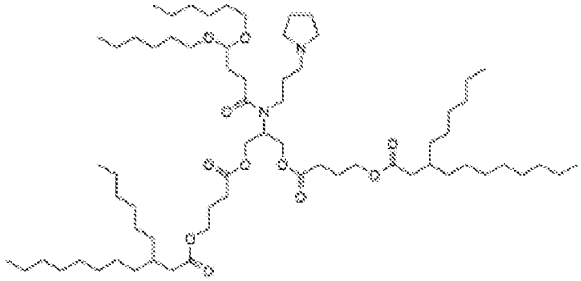
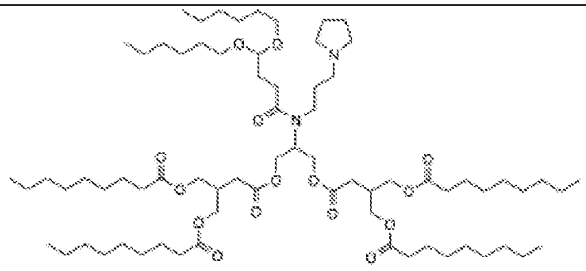
A-26		2-(N-(3-(cyclopropyl(2-hydroxyethyl)amino)propyl)-4-(decan-4-yloxy)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)
A-27		2-((3-(dimethylamino)propyl)(pentyl)amino)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)

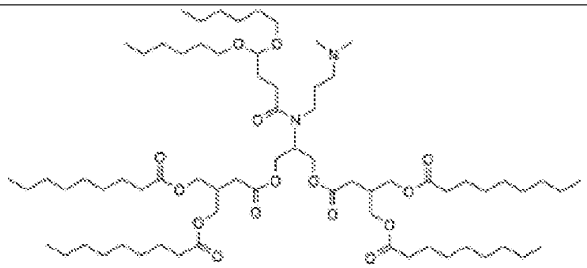
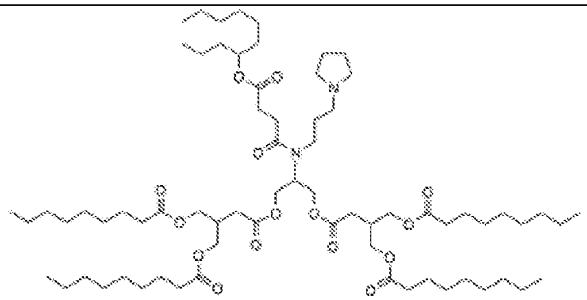
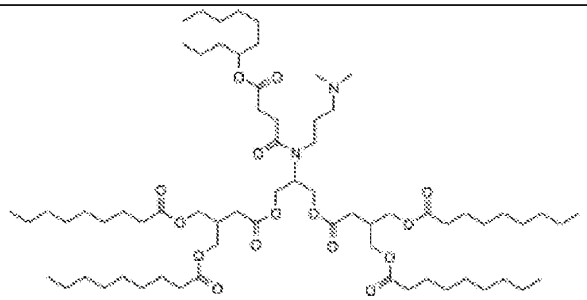
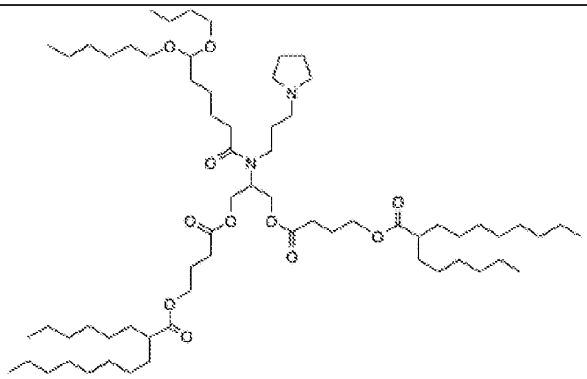
A-28		2-(cyclohexyl(3-((2-hydroxyethyl)(methyl)amino)propyl)amino)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)
A-29		2-((3-(cyclopropyl(2-hydroxyethyl)amino)propyl)(methyl)amino)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)

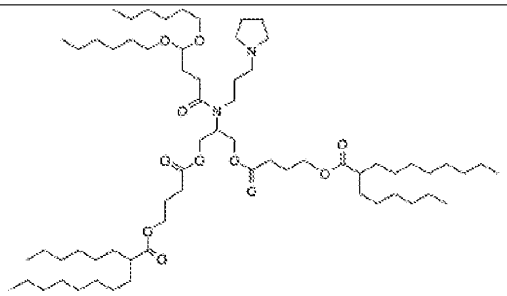
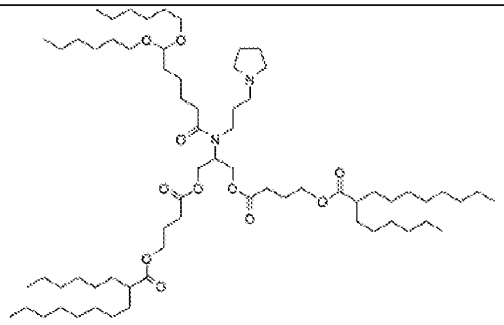
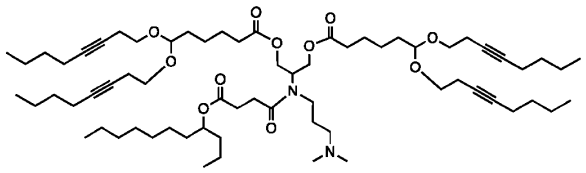
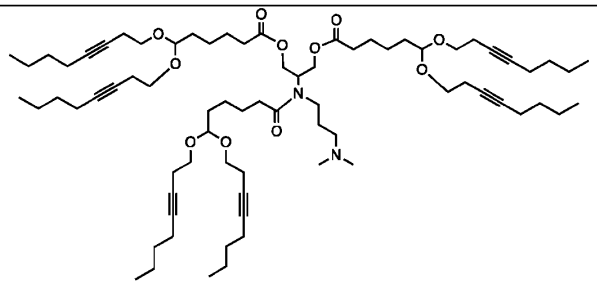
A-30		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-31		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-32		2-(4-(decan-4-yloxy)-4-oxo-N-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)

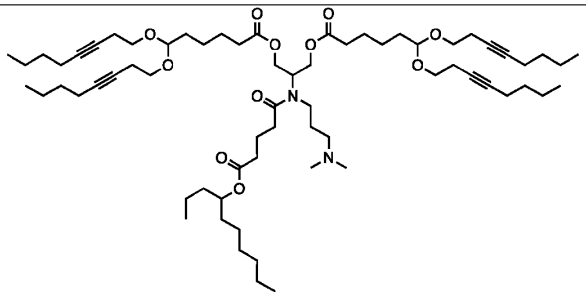
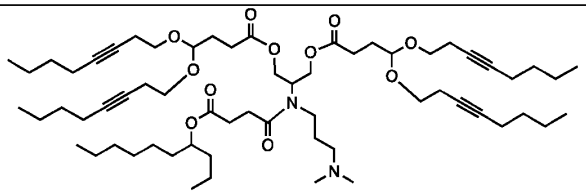
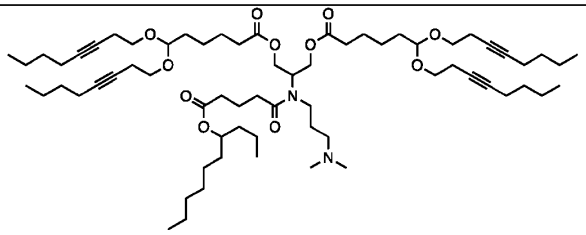
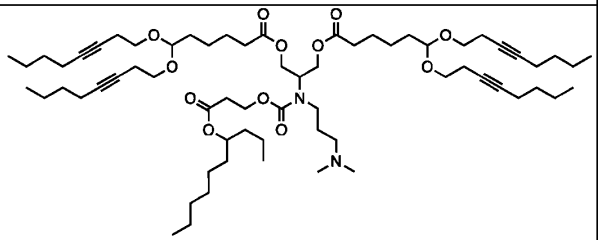
A-33		2-(4-(decan-4-yloxy)-4-oxo-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl bis(6,6-bis(((*Z*)-oct-5-en-1-yl)oxy)hexanoate)
A-34		2-(4-(decan-4-yloxy)-4-oxo-*N*-(3-(piperidin-1-yl)propyl)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-35		2-(4-(decan-4-yloxy)-4-oxo-*N*-(2-(pyrrolidin-1-yl)ethyl)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)

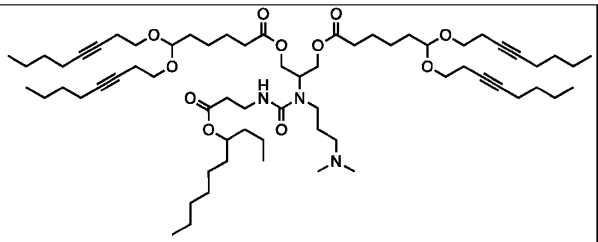
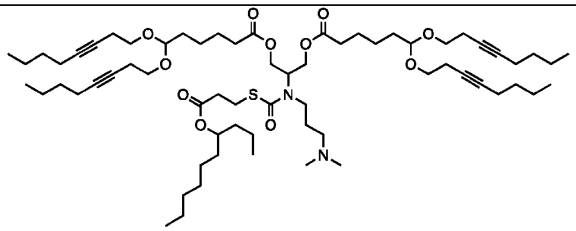
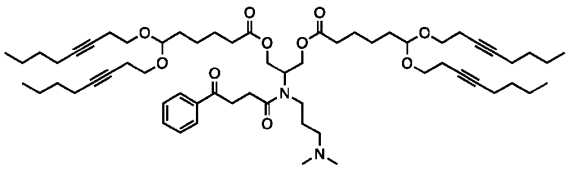
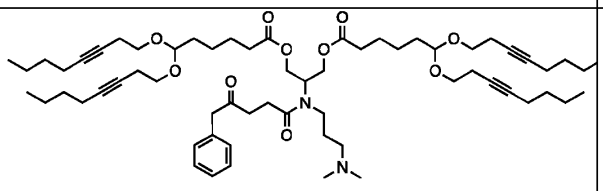
A-36		2-(4-(decan-4-yloxy)-4-oxo-*N*-(4-(pyrrolidin-1-yl)butyl)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-40		((2-(4,4-dibutoxy-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(2-hexyldecanoate)
A-41		((2-(6,6-dibutoxy-*N*-(3-(dimethylamino)propyl)hexanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(2-hexyldecanoate)

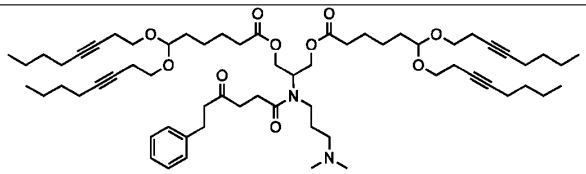
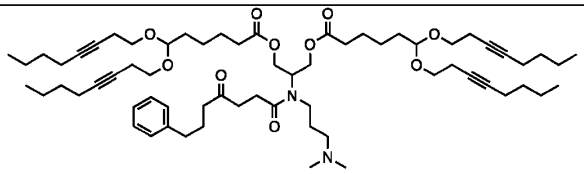
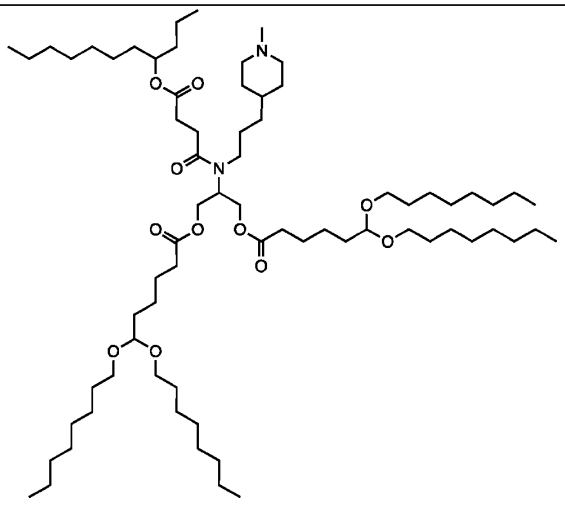
A-42		*O*,*O*'-(2-(4,4-bis(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl) di(pentadecan-7-yl) diglutarate
A-43		((2-(4,4-bis(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) dinonanoate
A-44		((2-(4,4-bis(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(3-hexylundecanoate)
A-45		(((2-(4,4-bis(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate

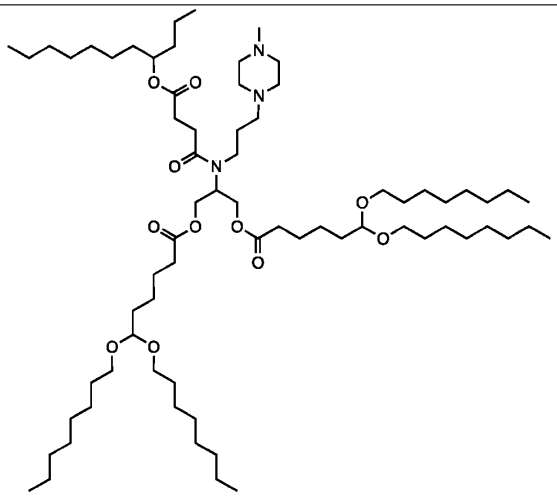
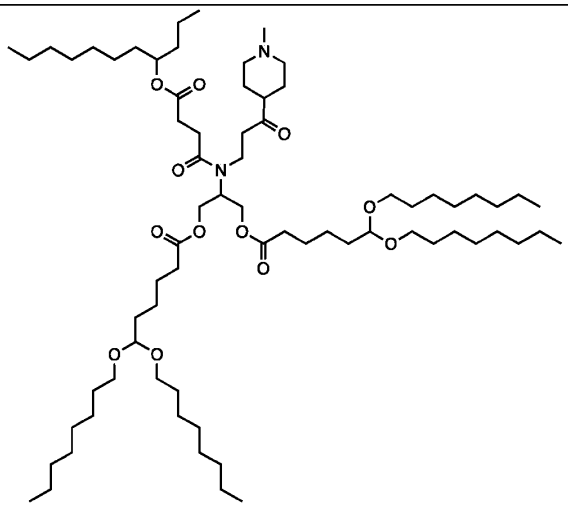
A-46		(((2-(*N*-(3-(dimethylamino)propyl)-4,4-bis(hexyloxy)butanamido)propane-1,3-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate
A-47		(((2-(4-(decan-4-yloxy)-4-oxo-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate
A-48		(((2-(4-(decan-4-yloxy)-*N*-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate
A-49		((2-(6-butoxy-6-(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)hexanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(2-hexyldecanoate)

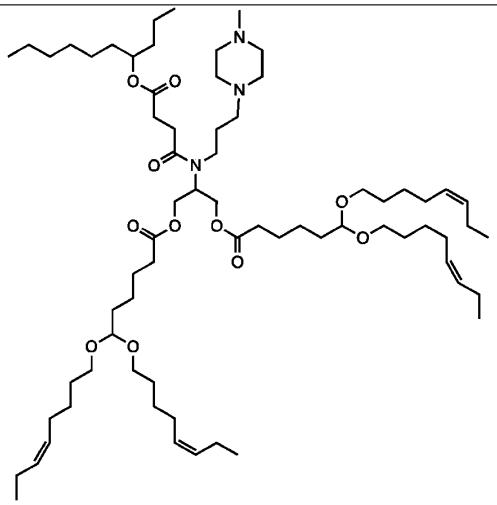
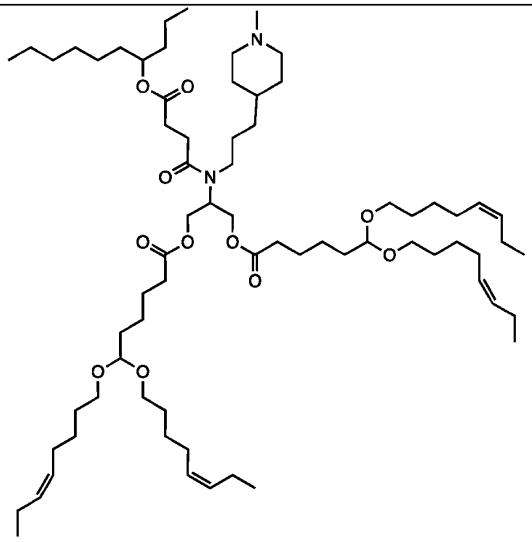
A-50		((2-(4,4-bis(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(2-hexyldecanoate)
A-51		((2-(6,6-bis(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)hexanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(2-hexyldecanoate)
A-52		2-(*N*-(3-(dimethylamino)propyl)-4-oxo-4-(undecan-4-yloxy)butanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-53		2-(*N*-(3-(dimethylamino)propyl)-6,6-bis(oct-3-yn-1-yloxy)hexanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)

A-54		2-(5-(decan-4-yloxy)-*N*-(3-(dimethylamino)propyl)-5-oxopentanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-55		2-(4-(decan-4-yloxy)-*N*-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(4,4-bis(oct-3-yn-1-yloxy)butanoate)
A-56		2-(5-(decan-4-yloxy)-*N*-(3-(dimethylamino)propyl)-5-oxopentanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-57		2-(((3-(decan-4-yloxy)-3-oxopropoxy)carbonyl)(3-(dimethylamino)propyl)amino)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)

A-58		2-(3-(3-(decan-4-yloxy)-3-oxopropyl)-1-(3-(dimethylamino)propyl)ureido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-59		2-((((3-(decan-4-yloxy)-3-oxopropyl)thio)carbonyl)(3-(dimethylamino)propyl)amino)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-60		2-(*N*-(3-(dimethylamino)propyl)-4-oxo-4-phenylbutanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-61		2-(*N*-(3-(dimethylamino)propyl)-4-oxo-5-phenylpentanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)

A-62		2-(*N*-(3-(dimethylamino)propyl)-4-oxo-6-phenylhexanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-63		2-(*N*-(3-(dimethylamino)propyl)-4-oxo-7-phenylheptanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-64		2-(*N*-(3-(1-methylpiperidin-4-yl)propyl)-4-oxo-4-(undecan-4-yloxy)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)

A-65		2-(*N*-(3-(4-methylpiperazin-1-yl)propyl)-4-oxo-4-(undecan-4-yloxy)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-66		2-(*N*-(3-(1-methylpiperidin-4-yl)-3-oxopropyl)-4-oxo-4-(undecan-4-yloxy)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)

A-67		2-(4-(decan-4-yloxy)-*N*-(3-(4-methylpiperazin-1-yl)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((*Z*)-oct-5-en-1-yl)oxy)hexanoate)
A-68		2-(4-(decan-4-yloxy)-*N*-(3-(1-methylpiperidin-4-yl)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((*Z*)-oct-5-en-1-yl)oxy)hexanoate)

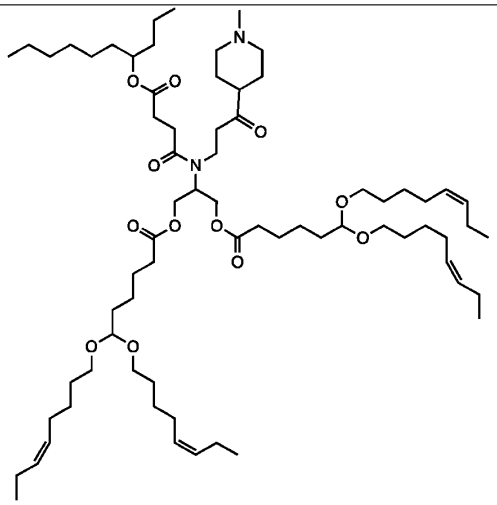
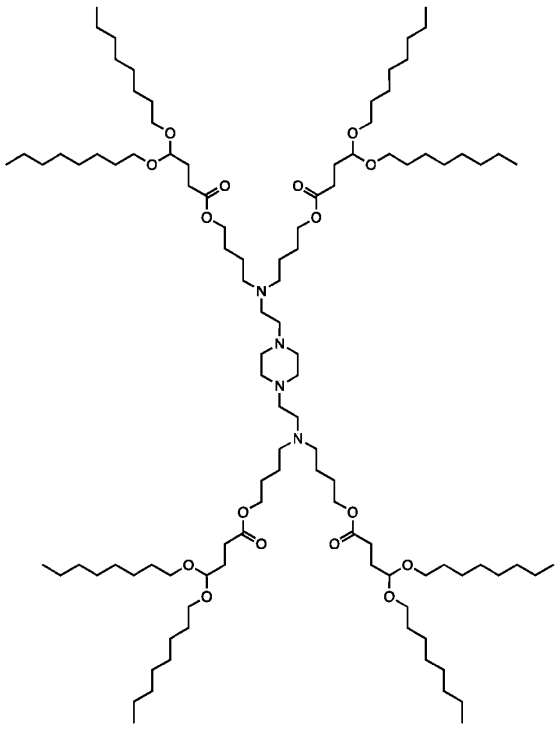
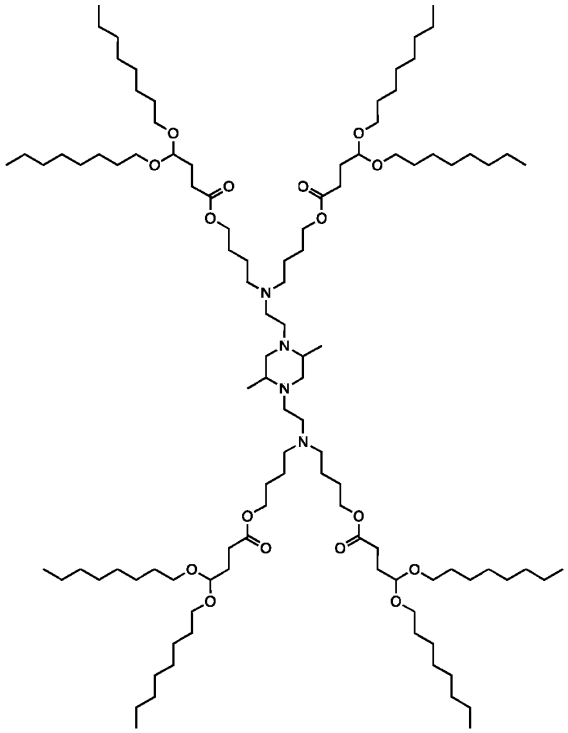
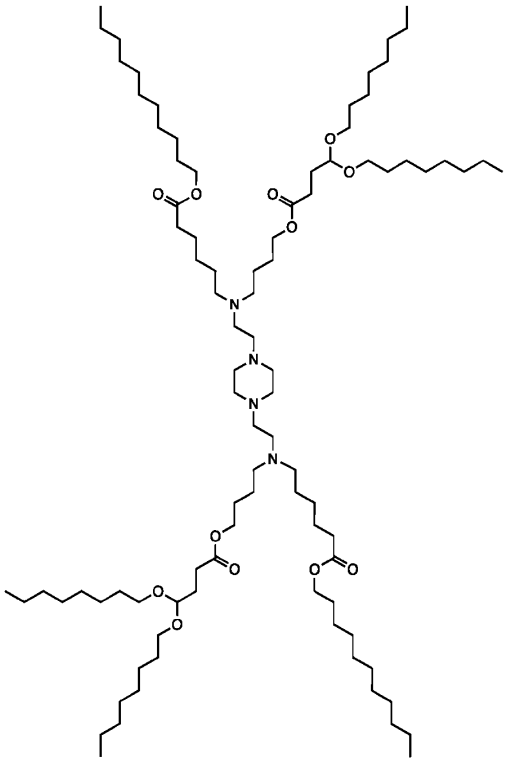
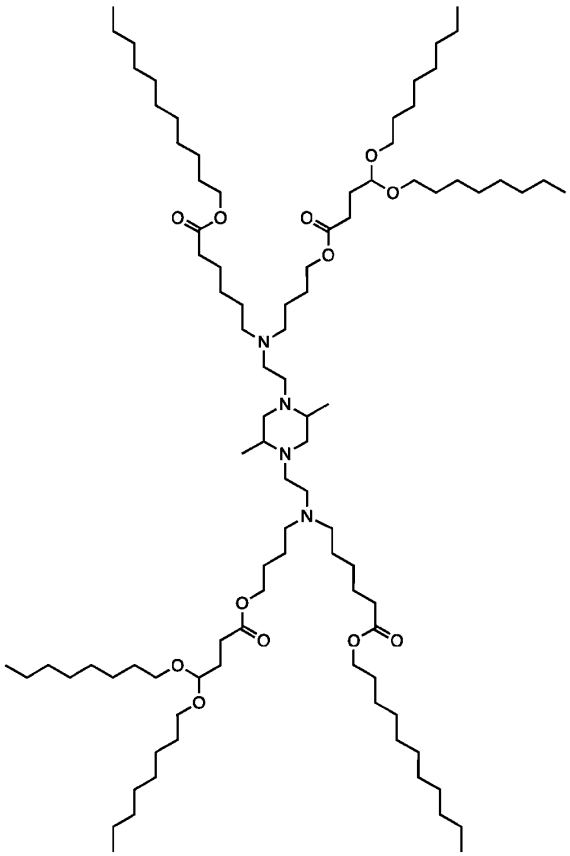
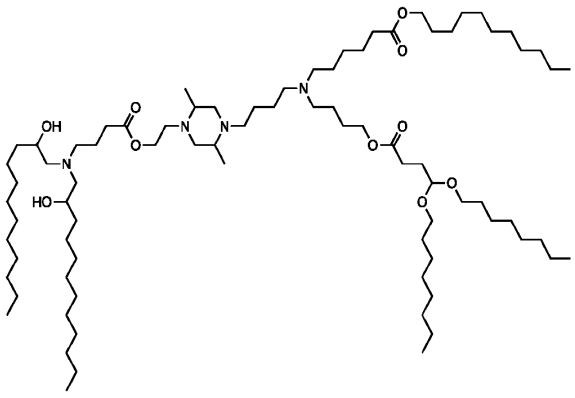
A-69		2-(4-(decan-4-yloxy)-*N*-(3-(1-methylpiperidin-4-yl)-3-oxopropyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((*Z*)-oct-5-en-1-yl)oxy)hexanoate)

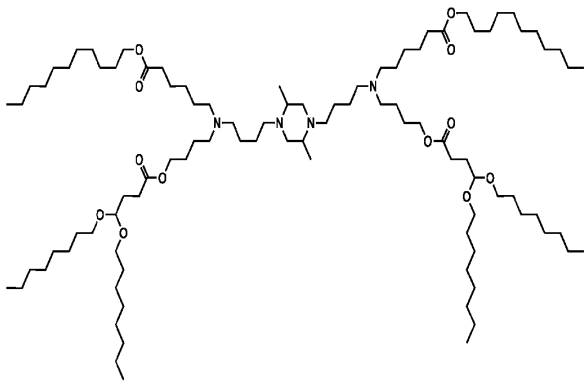
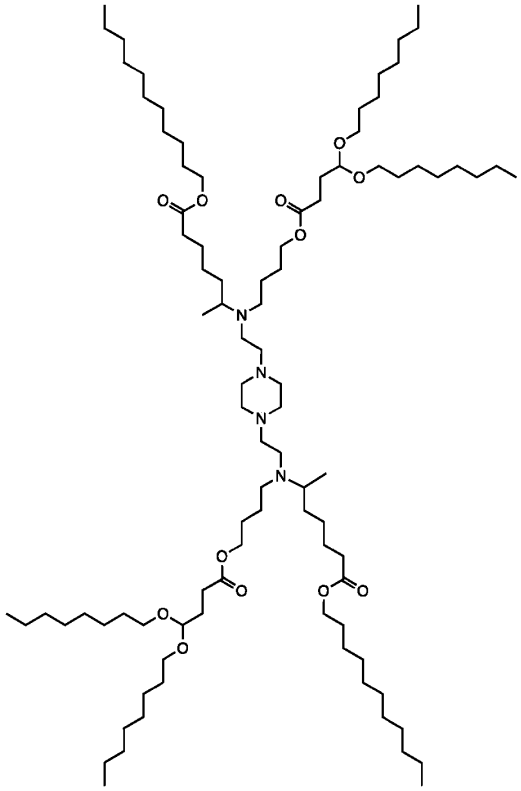
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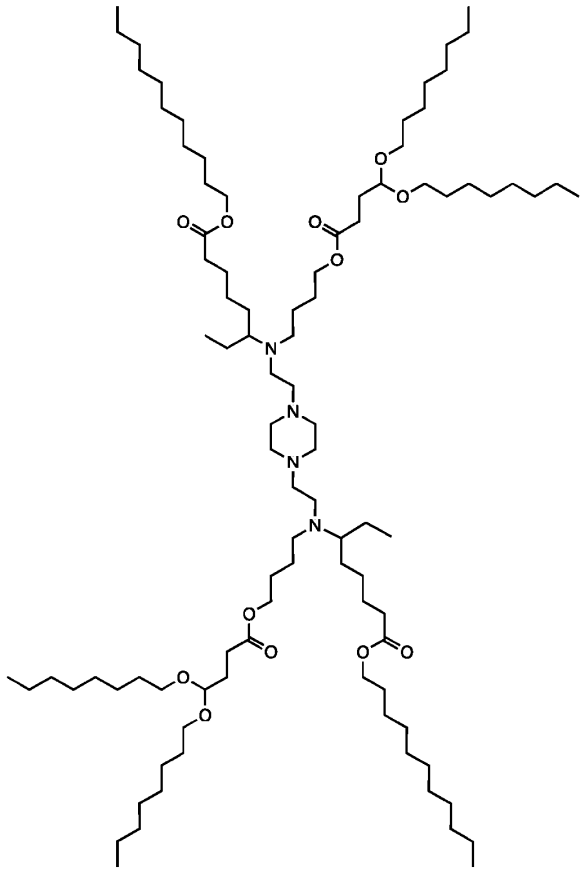
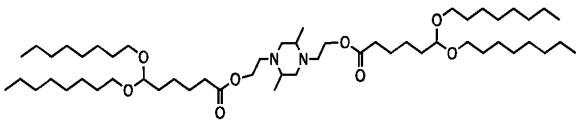
Compound	Structure	Name
B-1		((piperazine-1,4-diylbis(ethane-2,1-diyl))bis(azanetriyl))tetrakis(butane-4,1-diyl) tetrakis(4,4-bis(octyloxy)butanoate)

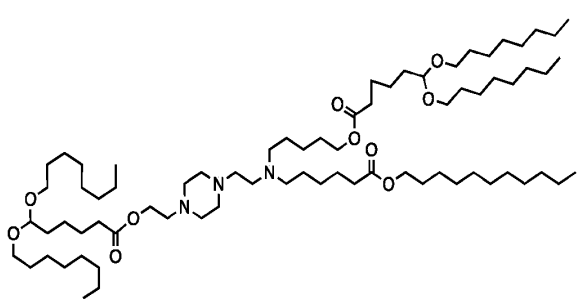
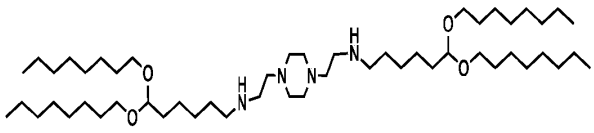
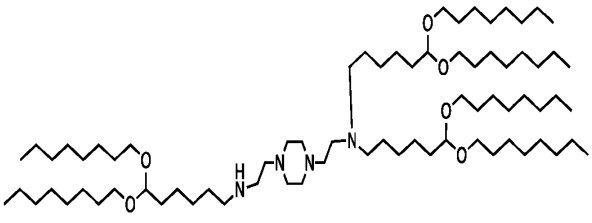
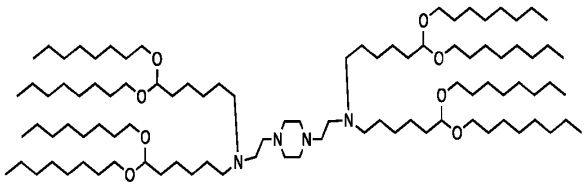
B-2		(((2,5-dimethylpiperazine-1,4-diyl)bis(ethane-2,1-diyl))bis(azanetriyl))tetrakis(butane-4,1-diyl) tetrakis(4,4-bis(octyloxy)butanoate)

B-3		diundecyl 6,6'-((piperazine-1,4-diylbis(ethane-2,1-diyl))bis((4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)azanediyl))dihexanoate

B-4		diundecyl 6,6'-(((2,5-dimethylpiperazine-1,4-diyl)bis(ethane-2,1-diyl))bis((4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)azanediyl))dihexanoate
B-5		undecyl 6-((4-(4-(2-((4-(bis(2-hydroxydodecyl)amino)butanoyl)oxy)ethyl)-2,5-dimethylpiperazin-1-yl)butyl)(4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)amino)hexanoate

B-6		diundecyl 6,6'-(((2,5-dimethylpiperazine-1,4-diyl)bis(butane-4,1-diyl))bis((4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)azanediyl))dihexanoate
B-7		diundecyl 6,6'-((piperazine-1,4-diylbis(ethane-2,1-diyl))bis((4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)azanediyl))diheptanoate

B-8		diundecyl 6,6'-((piperazine-1,4-diylbis(ethane-2,1-diyl))bis((4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)azanediyl))dioctanoate
B-9		(2,5-dimethylpiperazine-1,4-diyl)bis(ethane-2,1-diyl) bis(6,6-bis(octyloxy)hexanoate)

B-10		5-((2-(4-(2-((6,6-bis(octyloxy)hexanoyl)oxy)ethyl)piperazin-1-yl)ethyl)(6-oxo-6-(undecyloxy)hexyl)amino)pentyl 6,6-bis(octyloxy)hexanoate
B-11		N,N'-(piperazine-1,4-diylbis(ethane-2,1-diyl))bis(6,6-bis(octyloxy)hexan-1-amine)
B-12		N-(6,6-bis(octyloxy)hexyl)-N-(2-(4-(2-((6,6-bis(octyloxy)hexyl)amino)ethyl)piperazin-1-yl)ethyl)-6,6-bis(octyloxy)hexan-1-amine
B-13		N,N'-(piperazine-1,4-diylbis(ethane-2,1-diyl))bis(N-(6,6-bis(octyloxy)hexyl)-6,6-bis(octyloxy)hexan-1-amine)

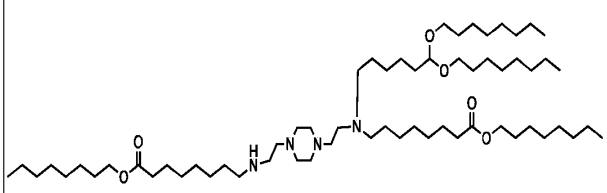
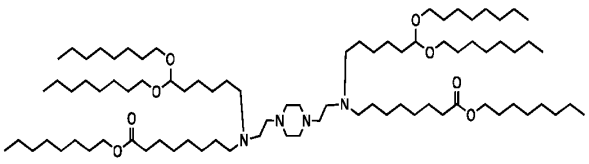
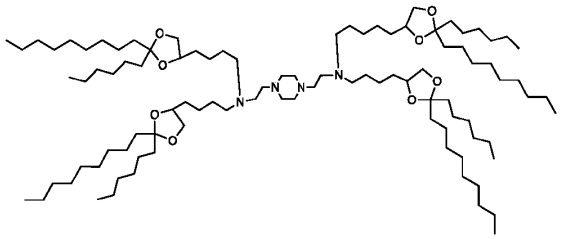
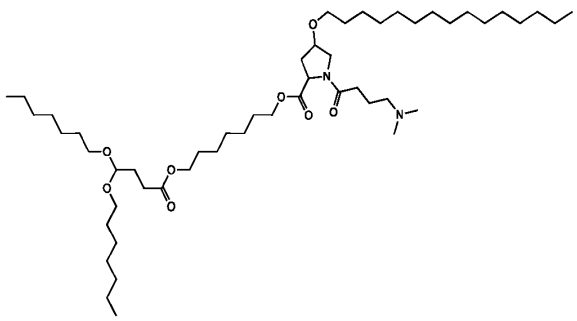
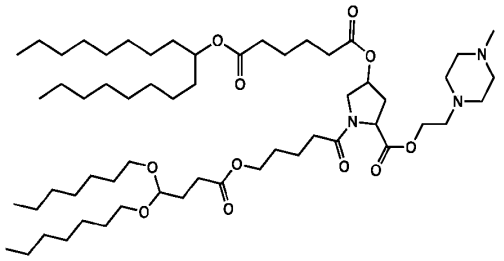
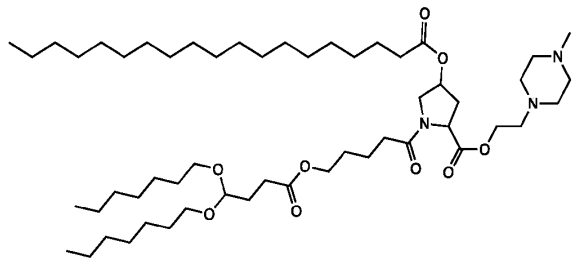
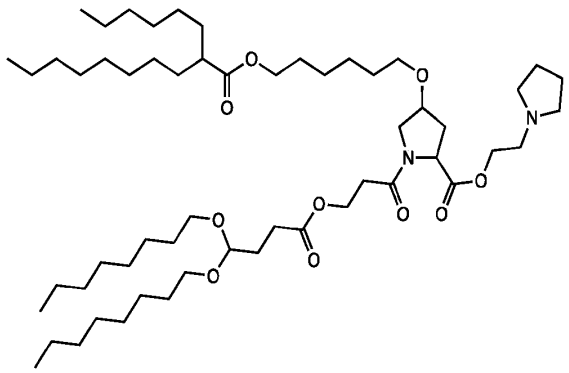
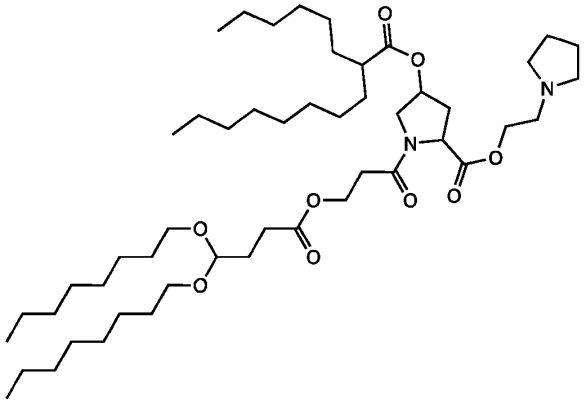
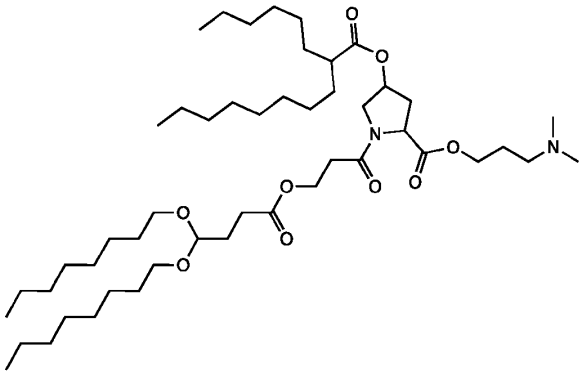
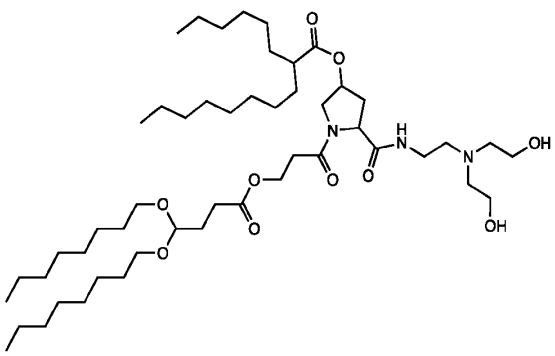
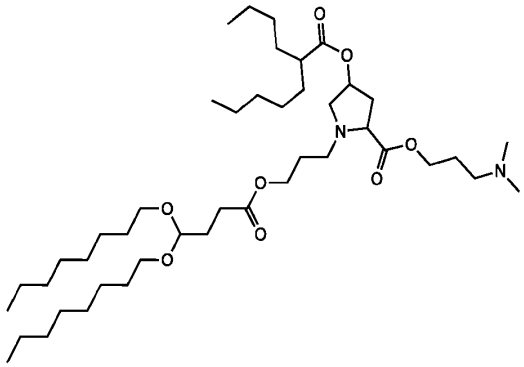
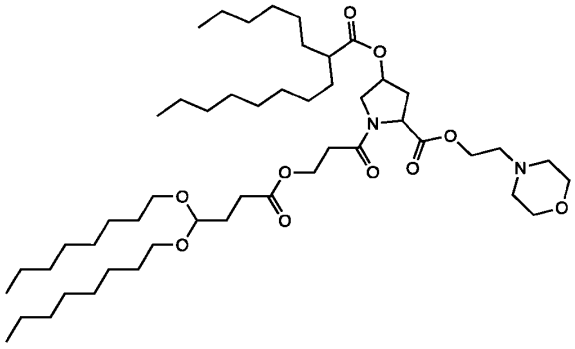
B-14		octyl 8-((6,6-bis(octyloxy)hexyl)(2-(4-(2-((8-(octyloxy)-8-oxooctyl)amino)ethyl)piperazin-1-yl)ethyl)amino)octanoate
B-15		dioctyl 8,8'-((piperazine-1,4-diylbis(ethane-2,1-diyl))bis((6,6-bis(octyloxy)hexyl)azanediyl))dioctanoate
B-16		N-(2-(4-(2-(bis(4-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)butyl)amino)ethyl)piperazin-1-yl)ethyl)-5-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)-N-(4-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)butyl)pentan-1-amine

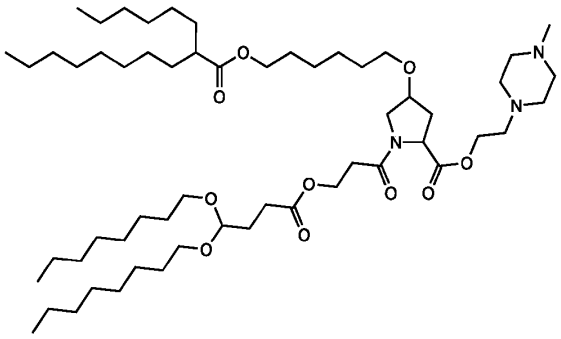
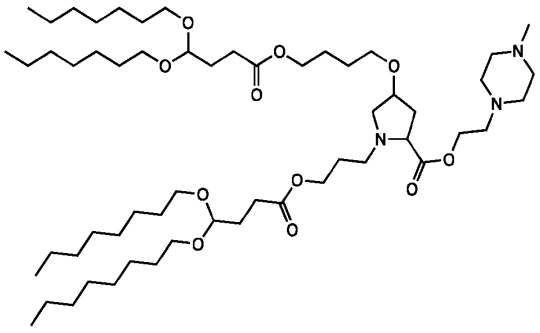
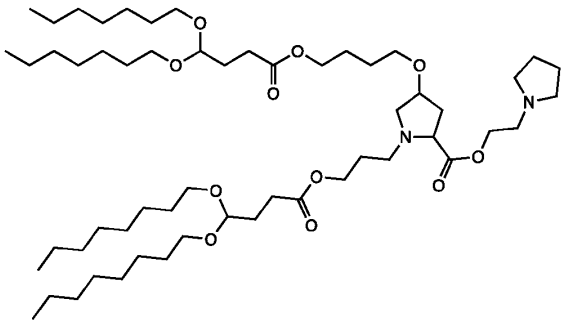
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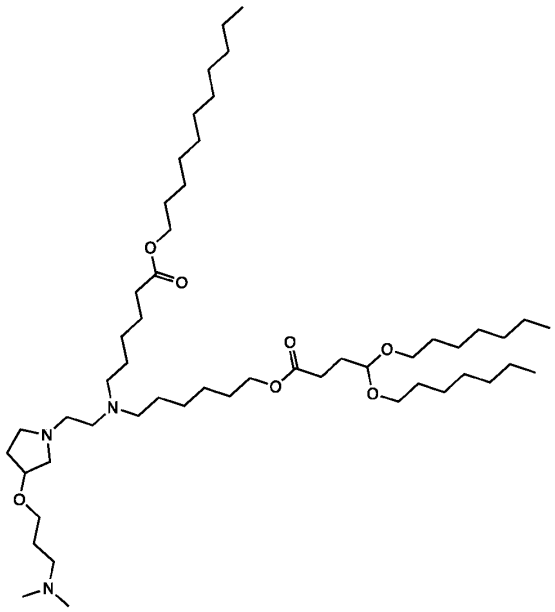
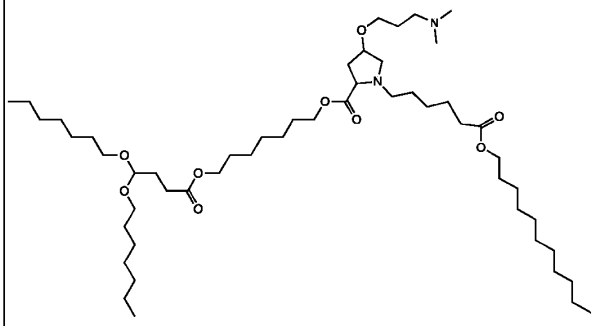
Compound	**Structure**	**Name**

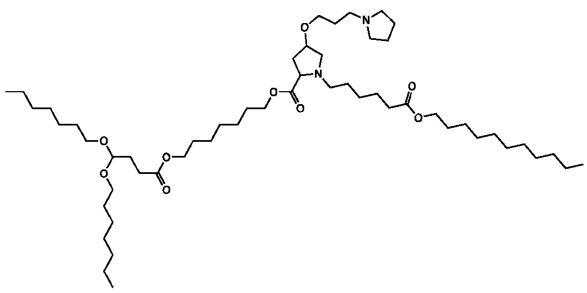
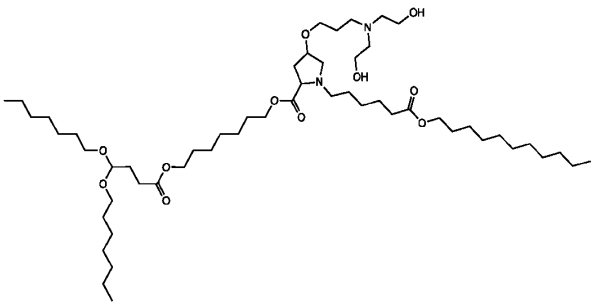
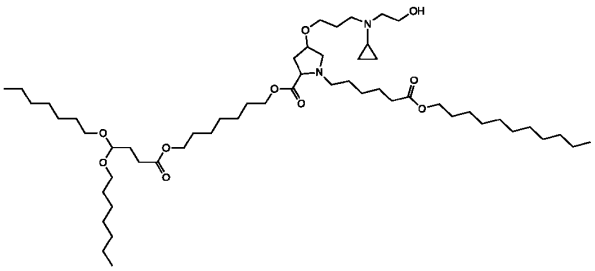
C-1		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 1-(4-(dimethylamino)butanoyl)-4-(pentadecyloxy)pyrrolidine-2-carboxylate
C-2		1-(5-((4,4-bis(heptyloxy)butanoyl)oxy)penta oyl)-5-((2-(4-methylpiperazin-1-yl)ethoxy)carbonyl)pyrrolidin-3-yl heptadecan-9-yl adipate
C-3		2-(4-methylpiperazin-1-yl)ethyl 1-(5-((4,4-bis(heptyloxy)butanoyl)oxy)penta oyl)-4-(nonadecanoyloxy)pyrrolidine-2-carboxylate

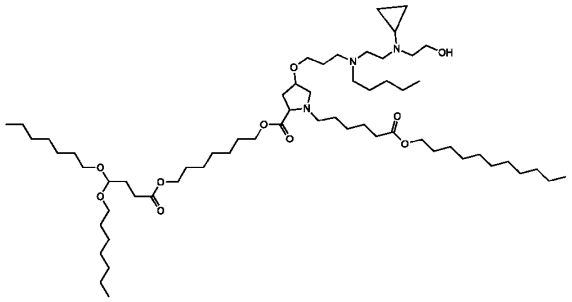
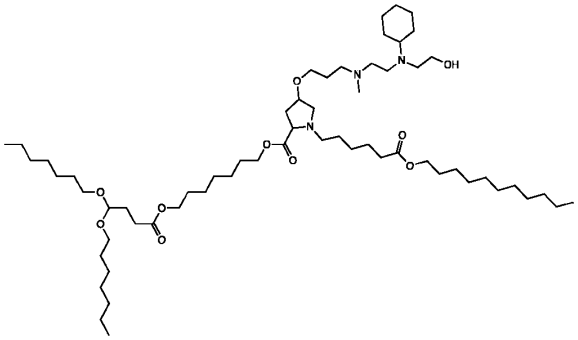
C-4		2-(pyrrolidin-1-yl)ethyl 1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propanyl)-4-((6-((2-hexyldecanoyl)oxy)hexyl)oxy)pyrrolidine-2-carboxylate
C-5		2-(pyrrolidin-1-yl)ethyl 1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propanyl)-4-((2-hexyldecanoyl)oxy)pyrrolidine-2-carboxylate
C-6		3-(dimethylamino)propyl 1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propanyl)-4-((2-hexyldecanoyl)oxy)pyrrolidine-2-carboxylate

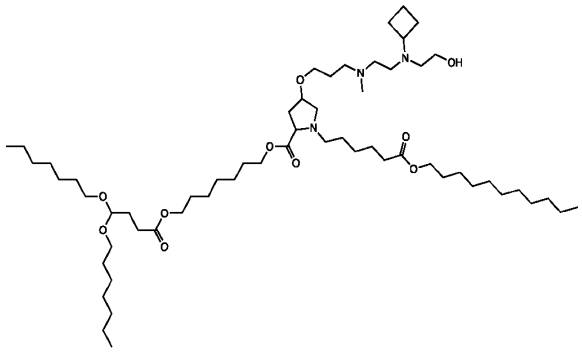
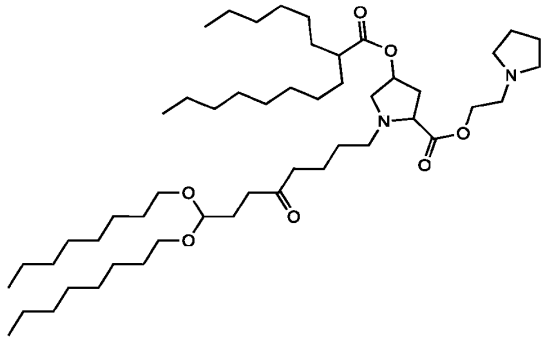
C-7		5-((2-(bis(2-hydroxyethyl)amino)ethyl)carbamyl)-1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propanyl)pyrrolidin-3-yl 2-hexyldecanoat
C-8		3-(dimethylamino)propyl 1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propyl)4-((2-butylheptanoyl)oxy)pyrrolidine-2-carboxylate
C-9		2-morpholinoethyl 1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propanyl)-4-((2-hexyldecanoyl)oxy)pyrrolidine-2-carboxylate

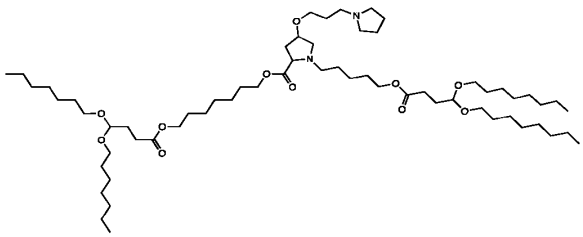
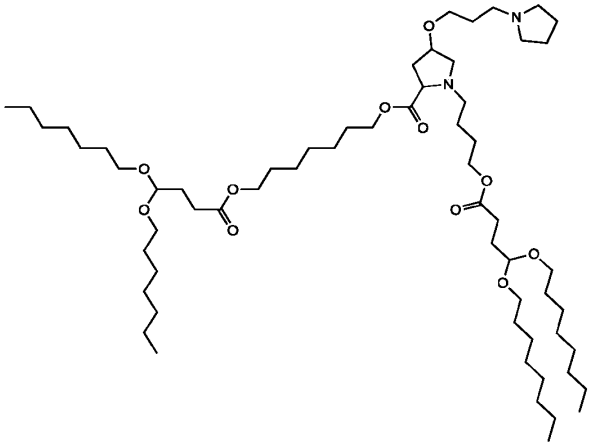
C-10		2-(4-methylpiperazin-1-yl)ethyl 1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propanyl)-4-((6-((2-hexyldecanoyl)oxy)hexyl)oxy)pyrolidine-2-carboxylate
C-11		2-(4-methylpiperazin-1-yl)ethyl 4-(4-((4,4-bis(heptyloxy)butanoyl)oxy)butox)-1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propyl)yrrolidine-2-carboxylate
C-12		2-(pyrrolidin-1-yl)ethyl 4-(4-((4,4-bis(heptyloxy)butanoyl)oxy)butox)-1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propyl)yrrolidine-2-carboxylate

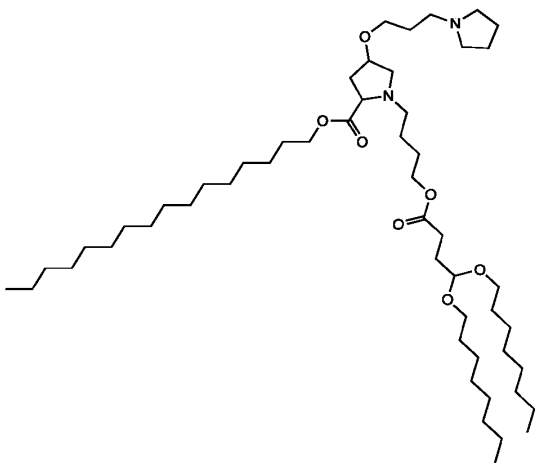
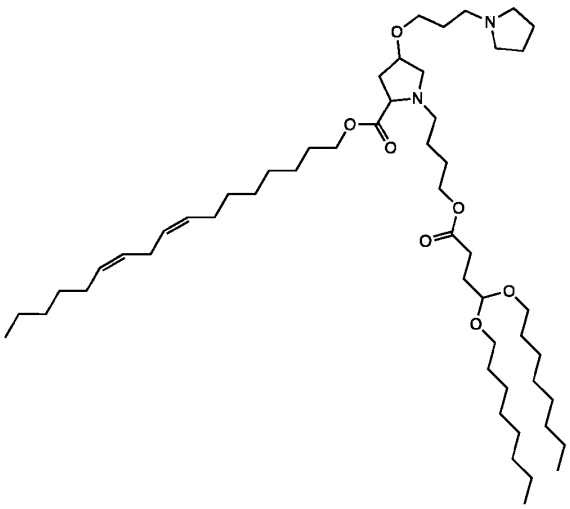
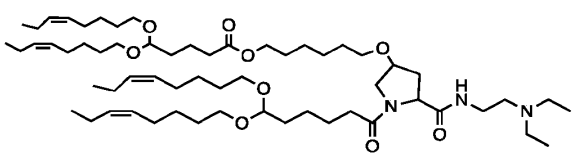
C-13		undecyl 6-((6-((4,4-bis(heptyloxy)butanoyl)oxy)hexyl 2-(3-(3-(dimethylamino)propoxy)pyrrolidi -1-yl)ethyl)amino)hexanoate
C-14		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 4-(3-(dimethylamino)propoxy)-1-(6-oxo-6-(undecyloxy)hexyl)pyrrolidine-2-carboxylate

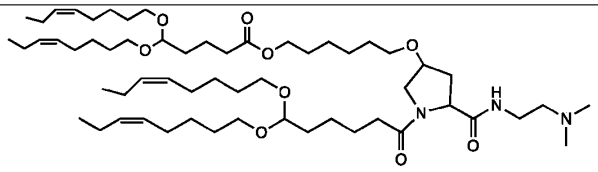
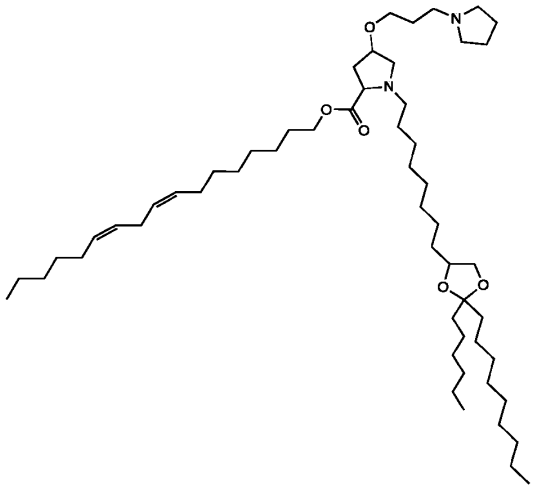
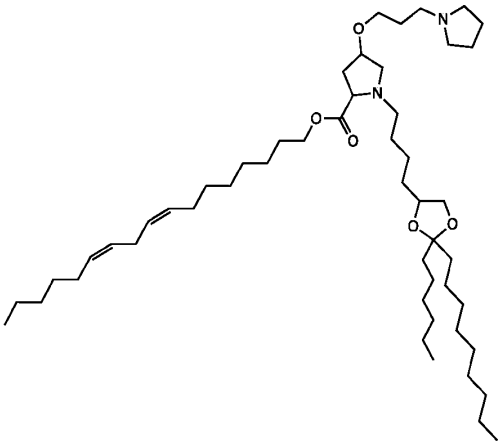
C-15		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 1-(6-oxo-6-(undecyloxy)hexyl)-4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate
C-16		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 4-(3-(bis(2-hydroxyethyl)amino)propoxy)-1-(oxo-6-(undecyloxy)hexyl)pyrrolidine-2-carboxylate
C-17		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 4-(3-(cyclopropyl(2-hydroxyethyl)amino)propoxy)-1-(oxo-6-(undecyloxy)hexyl)pyrrolidine-2-carboxylate

C-18		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 4-(3-((2-(cyclopropyl(2-hydroxyethyl)amino)ethyl)(pentyl mino)propoxy)-1-(6-oxo-6-(undecyloxy)hexyl)pyrrolidine-2-carboxylate
C-19		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 4-(3-((2-(cyclohexyl(2-hydroxyethyl)amino)ethyl)(methyl amino)propoxy)-1-(6-oxo-6-(undecyloxy)hexyl)pyrrolidine-2-carboxylate

C-20		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 4-(3-((2-(cyclobutyl(2-hydroxyethyl)amino)ethyl)(methyl amino)propoxy)-1-(6-oxo-6-(undecyloxy)hexyl)pyrrolidine-2-carboxylate
C-21		2-(pyrrolidin-1-yl)ethyl 1-(8,8-bis(octyloxy)-5-oxooctyl)-4-((2-hexyldecanoyl)oxy)pyrrolidine-2-carboxylate

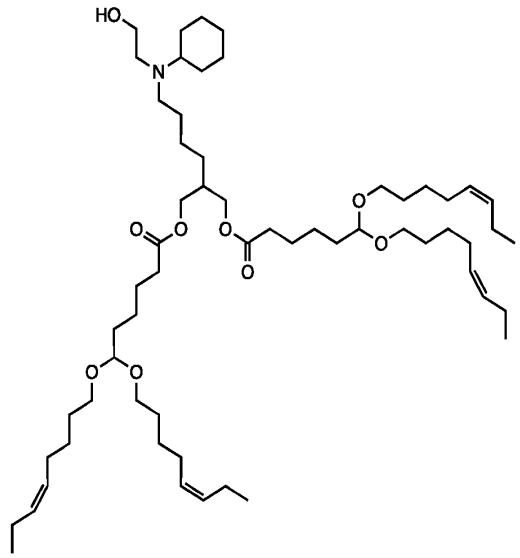
C-22		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 1-(5-((4,4-bis(octyloxy)butanoyl)oxy)pentyl) 4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate
C-23		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 1-(4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)- 4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate

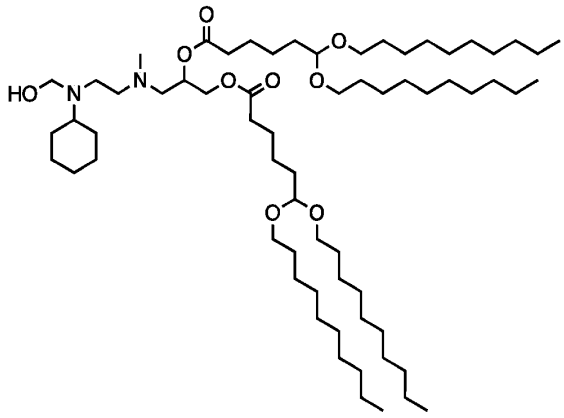
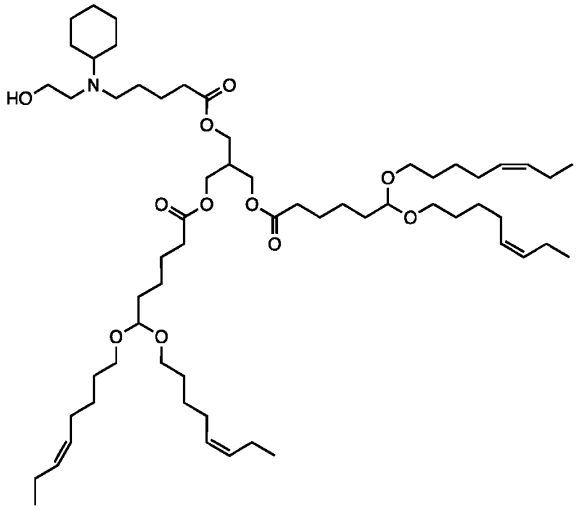
C-24		hexadecyl 1-(4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)-4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate
C-25		(8Z,11Z)-heptadeca-8,11-dien-1-yl 1-(4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)-4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate
C-26-1		6-((1-(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoyl)-5-((2-(diethylamino)ethyl)carbamoyl)pyrrolidin-3-yl)oxy)hexyl 5,5-bis(((Z)-oct-5-en-1-yl)oxy)pentanoate

C-26		6-((1-(6,6-bis(((*Z*)-oct-5-en-1-yl)oxy)hexanoyl)-5-((2-(dimethylamino)ethyl)carbamoyl)pyrrolidin-3-yl)oxy)hexyl 5,5-bis(((*Z*)-oct-5-en-1-yl)oxy)pentanoate
C-27		(8Z,11Z)-heptadeca-8,11-dien-1-yl 1-(8-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)octyl)-4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate
C-28		(8Z,11Z)-heptadeca-8,11-dien-1-yl 1-(4-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)butyl)-4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate

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Table D

Compound	Structure	Name
D-1		2-(4-(cyclohexyl(2-hydroxyethyl)amino)butyl)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)

D-2		3-((2-(cyclohexyl(hydroxymethyl)amino)ethyl)(methyl)amino)propane-1,2-diyl bis(6,6-bis(decyloxy)hexanoate)
D-3		2-(((5-(cyclohexyl(2-hydroxyethyl)amino)pentanoyl)oxy)methyl)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)

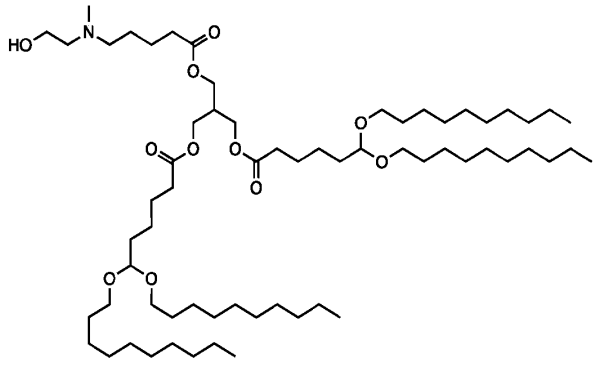
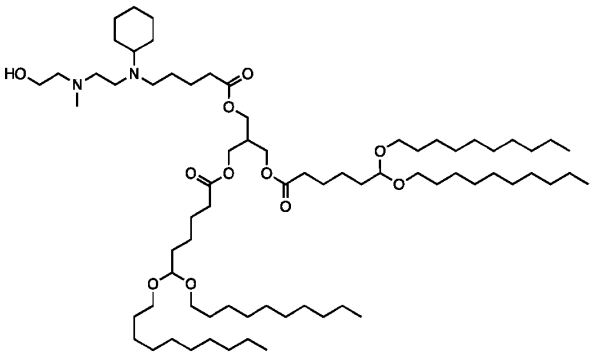
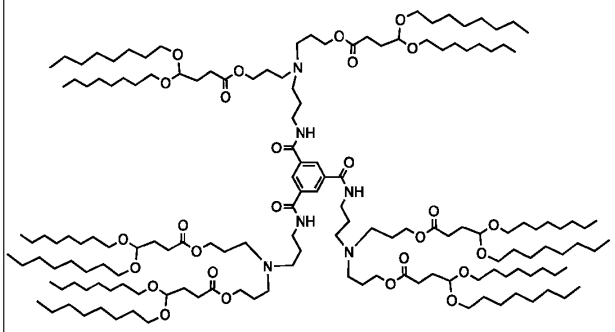
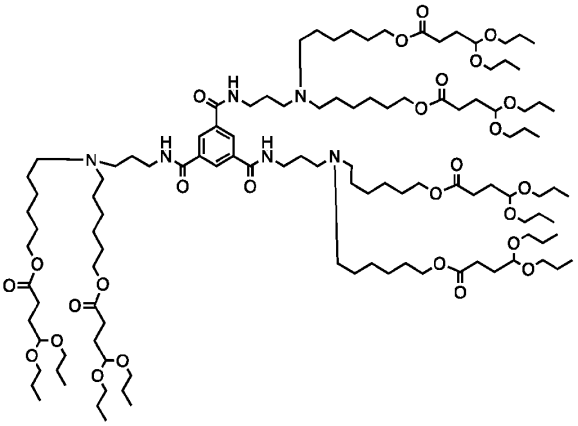
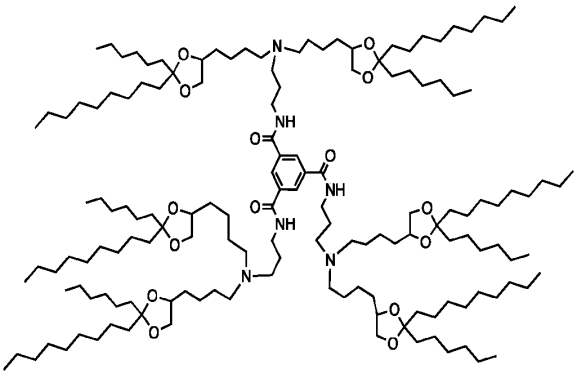
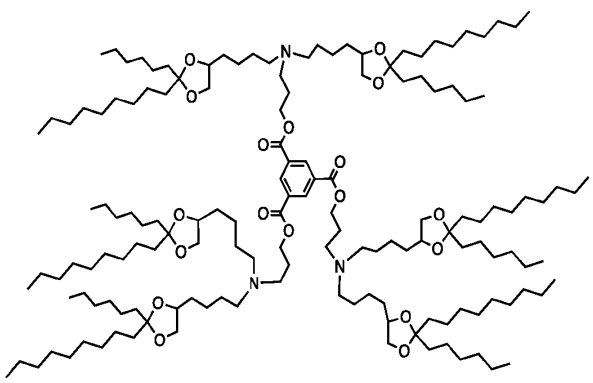
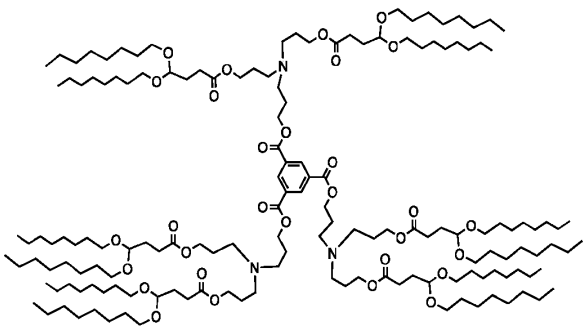
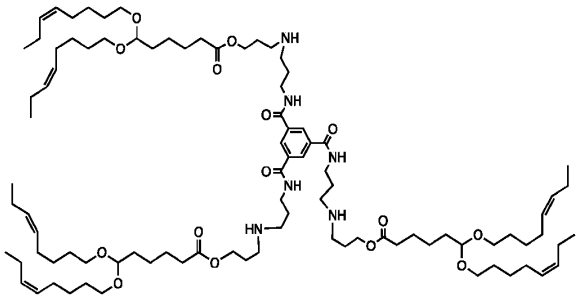
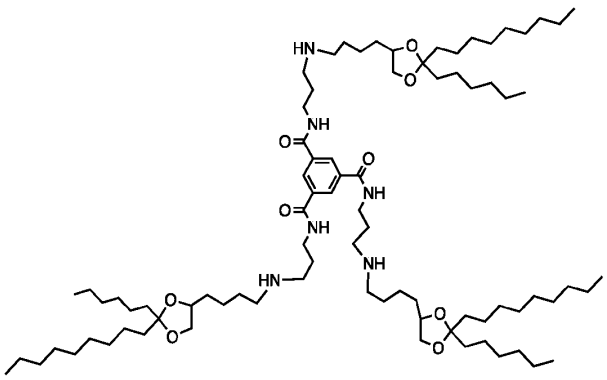
D-4		2-(((5-((2-hydroxyethyl)(methyl)amino)pentanoyl)oxy)methyl)propane-1,3-diyl bis(6,6-bis(decyloxy)hexanoate)
D-5		2-(((5-(cyclohexyl(2-((2-hydroxyethyl)(methyl)amino)ethyl)amino)pentanoyl)oxy)methyl)propane-1,3-diyl bis(6,6-bis(decyloxy)hexanoate)

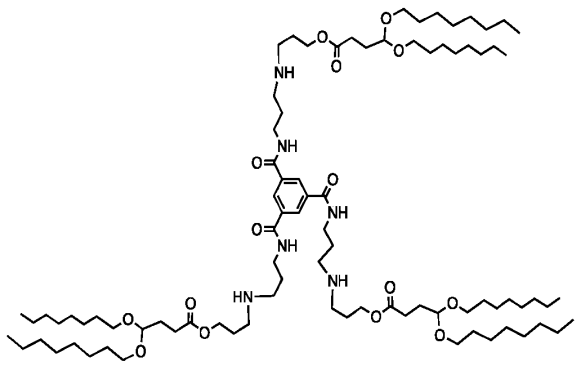
Table E

Compound	**Structure**	**Name**

E-1		((((benzene-1,3,5-tricarbonyl)tris(azanediyl))tris(propane-3,1-diyl))tris(azanetriyl))hexakis(propane-3,1-diyl) hexakis(4,4-bis(octyloxy)butanoate)
E-2		((((benzene-1,3,5-tricarbonyl)tris(azanediyl))tris(propane-3,1-diyl))tris(azanetriyl))hexakis(hexane-6,1-diyl) hexakis(4,4-dipropoxybutanoate)
E-3		N1,N3,N5-tris(3-(bis(4-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)butyl)amino)propyl)benzene-1,3,5-tricarboxamide

E-4		tris(3-(bis(4-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)butyl)amino)propyl) benzene-1,3,5-tricarboxylate
E-5		tris(3-(bis(3-((4,4-bis(octyloxy)butanoyl)oxy)propyl)amino)propyl) benzene-1,3,5-tricarboxylate

E-6		((((benzene-1,3,5-tricarbonyl)tris(azanediyl))tris(propane-3,1-diyl))tris(azanediyl))tris(propane-3,1-diyl) tris(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)
E-7		N1,N3,N5-tris(3-((4-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)butyl)amino)propyl)benzene-1,3,5-tricarboxamide

E-8		((((benzene-1,3,5-tricarbonyl)tris(azanediyl))tris(propane-3,1-diyl))tris(azanediyl))tris(propane-3,1-diyl) tris(4,4-bis(octyloxy)butanoate)

Also provided herein is a lipid nanoparticle (LNP) comprising a lipid blend comprising an ionizable cationic lipid and/or lipid-immune cell targeting group conjugate (e.g., a lipid-T-cell targeting group conjugate) provided herein.

In some embodiments, the lipid blend comprises one or more of a structural lipid (e.g., a sterol), a neutral phospholipid, and a free PEG-lipid.

In some embodiments, the LNP comprises an ionizable lipid of the invention, distearoylphosphatidylcholine (DSPC), cholesterol, and 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2000), optionally at a molar ratio of about 48.5:10:40:1.5, respectively.

In some embodiments, the LNP comprises an ionizable lipid of the invention, distearoylphosphatidylcholine (DSPC), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000), optionally at a molar ratio of about 48.5:10:40:1.5, respectively.

In some embodiments, the LNP comprises an ionizable lipid of the invention, distearoylphosphatidylcholine (DSPC), cholesterol, and 1,2-dimyristoyl-rac-glycero-3-

methoxypolyethylene glycol-2000 (DMG-PEG2000), optionally at a molar ratio of about 48.5:10:39:2.5, respectively.

The amounts and ratios of LNP components may be varied by any amount dependent on the desired form, structure, function, cargo, target, or any combination thereof. The amount of each component may be expressed in various embodiments as percent of the total molar mass of all lipid or lipid conjugated components accounted for by the indicated component (mol%). The amount of each component may be expressed in various embodiments as the relative ratio of each component based on molar mass (Molar Ratio). The amount of each component may be expressed in various embodiments as the weight of each component used to formulate the LNP prior to fabrication (mg or equivalent). The amount of each component may be expressed in various embodiments by any other method known in the art. Any formulation given in one representation of component amounts ("units") is expressly meant to encompass any formulation expressed in different units of component amounts, wherein those representations are effectively equivalent when converted into the same units. In some embodiments, "effectively equivalent" means two or more values within about 10% of one another.

In some aspects, provided are methods of expressing a polypeptide of interest in a targeted cell, e.g., immune cell, of a subject. In some embodiments, the method comprises contacting the immune cell with a lipid nanoparticle (LNP) provided herein.

In some aspects, provided are methods of modulating cellular function of a target cell, e.g., target immune cell, of a subject. In some embodiments, the methods comprise administering to the subject a lipid nanoparticle (LNP) provided herein.

In some aspects, provided are methods of treating, ameliorating, or preventing a symptom of a disorder or disease in a subject in need thereof. In some embodiments, the methods comprise administering to the subject a lipid nanoparticle (LNP) provided herein.

Various aspects and embodiments of the invention are described in further detail below.

DETAILED DESCRIPTION

The invention provides ionizable cationic lipids and lipid nanoparticle compositions comprising such ionizable cationic lipids, medical kits containing such lipids, and methods of making and using, such lipids and conjugates. The compositions may further comprise lipid-immune cell targeting group conjugates.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of organic chemistry, pharmacology, cell biology, and biochemistry. Such techniques are explained in the literature, such as in "Comprehensive Organic Synthesis" (B.M. Trost & I. Fleming, eds., 1991-1992); "Current protocols in molecular biology" (F.M. Ausubel et al., eds., 1987, and periodic updates); and "Current protocols in immunology" (J.E. Coligan et al., eds., 1991), each of which is herein incorporated by reference in its entirety. Various aspects of the invention are set forth below in sections; however, aspects of the invention described in one particular section are not to be limited to any particular section.

I. DEFINITIONS

To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein should be construed according to the standard rules of chemical valency known in the chemical arts. In addition, when a chemical group is a diradical, for example, it is understood a that the chemical groups can be bonded to their adjacent atoms in the remainder of the structure in one or both orientations, for example, -OC(O)- is interchangeable with -C(O)O- or -OC(S)- is interchangeable with -C(S)O-.

The terms "cationic lipid" or "ionizable cationic lipid" are used interchangeably or together herein and refer to lipids that are protonated (e.g., >50% protonated) at low pH (e.g., pH 4), which makes them positively charged, but they may remain neutral at physiological pH (e.g., pH 7.4).

The terms "a" and "an" as used herein mean "one or more" and include the plural unless the context is inappropriate. In some embodiments, "one or more" is 1 or 2. In some embodiments, "one or more" is 1, 2, or 3. In some embodiments, "one or more" is 1, 2, 3, or 4. In some embodiments, "one or more" is 1, 2, 3, 4, or 5. In some embodiments, "one or more" is 1, 2, 3, 4, 5, or more.

The term "alkyl" as used herein refers to a saturated straight or branched hydrocarbon, such as a straight or branched group of 1-12, 1-10, or 1-6 carbon atoms, referred to herein as C_1-C_{12}alkyl, C_1-C_{10}alkyl, or C_1-C_6alkyl, respectively. In some embodiments, alkyl is optionally substituted. Exemplary alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, 2-methyl-1-propyl, 2-methyl-2-propyl, 2-methyl-1-butyl, 3-methyl-1-butyl, 2-methyl-3-butyl, 2,2-dimethyl-1-propyl, 2-methyl-1-pentyl, 3-methyl-1-pentyl, 4-methyl-1-pentyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 2,2-dimethyl-1-butyl, 3,3-dimethyl-1-butyl, 2-ethyl-1-butyl, butyl, isobutyl, t-butyl, pentyl, isopentyl, neopentyl, hexyl, heptyl, octyl, etc.

The term "alkylenyl" or "alkylene" refers to a diradical of an alkyl group. In some embodiments, alkylene is optionally substituted. An exemplary alkylene group is $-CH_2CH_2-$.

The term "alkenylenyl" or "alkenylene" refers to a diradical of an alkenyl group. In some embodiments, alkenylene is optionally substituted. An exemplary alkenylene group is –CH=CH-.

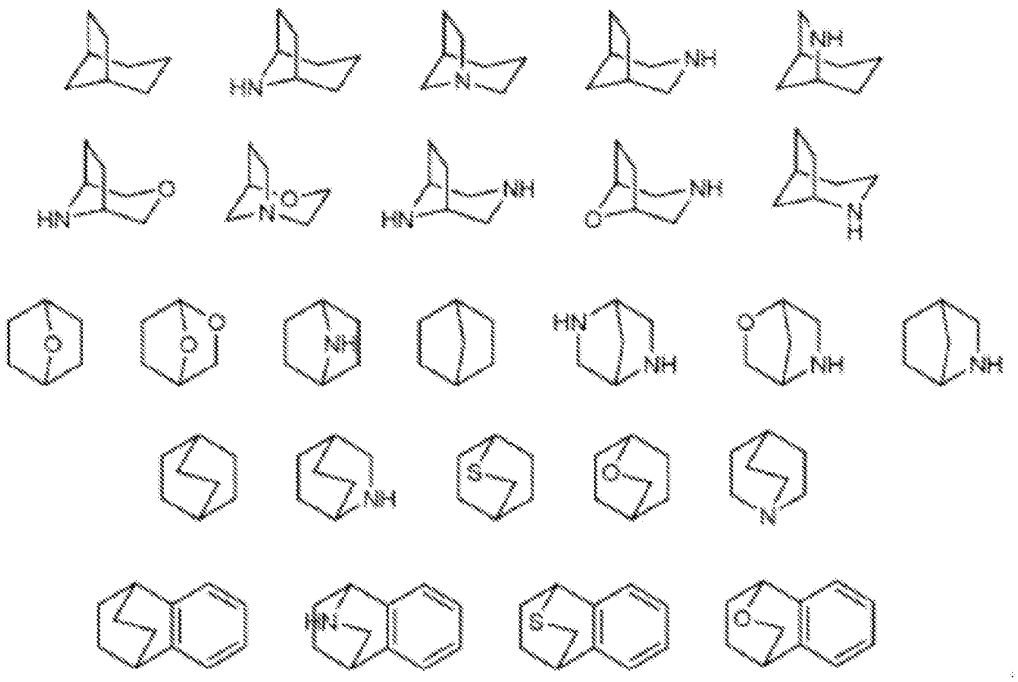
The term "akynylenyl" or "alkynylene" refers to a diradical of an alkenyl group. In some embodiments, alkynylene is optionally substituted. An exemplary alkynylene group is $-C\equiv C-$.

The term "haloalkyl" refers to an alkyl group that is substituted with at least one halogen. For example, $-CH_2F$, $-CHF_2$, $-CF_3$, $-CH_2CF_3$, $-CF_2CF_3$, and the like.

The term "aliphatic" or "aliphatic group", as used herein, means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation, or a monocyclic hydrocarbon or bicyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic (also referred to herein as "carbocycle," "carbocyclic", "cycloaliphatic" or "cycloalkyl"), that has a single point of attachment to the rest of the molecule. Unless otherwise specified, aliphatic groups contain 1-6 aliphatic carbon atoms. In

some embodiments, aliphatic groups contain 1-5 carbon atoms. In some embodiments, aliphatic groups contain 1-4 carbon atoms. In some embodiments, aliphatic groups contain 1-3 carbon atoms, and in some embodiments, aliphatic groups contain 1-2 carbon atoms. In some embodiments, "carbocyclic" (or "cycloaliphatic" or "carbocycle" or "cycloalkyl") refers to an optionally substituted monocyclic C_3-C_8 hydrocarbon, or an optionally substituted C_6-C_{12} bicyclic hydrocarbon, that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule. Suitable aliphatic groups include, but are not limited to, linear or branched, substituted or unsubstituted alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

The term "bridged bicyclic" refers to any bicyclic ring system, i.e., carbocyclic or heterocyclic, saturated or partially unsaturated, having at least one bridge. As defined by IUPAC, a "bridge" is an unbranched chain of atoms or an atom or a valence bond connecting two bridgeheads, where a "bridgehead" is any skeletal atom of the ring system which is bonded to three or more skeletal atoms (excluding hydrogen). In some embodiments, a bridged bicyclic group has 7-12 ring members and 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Such bridged bicyclic groups are well known in the art and include those groups set forth below where each group is attached to the rest of the molecule at any substitutable carbon or nitrogen atom. Unless otherwise specified, a bridged bicyclic group is optionally substituted with one or more substituents as set forth for aliphatic groups. Additionally or alternatively, any substitutable nitrogen of a bridged bicyclic group is optionally substituted. Exemplary bridged bicyclics include but are not limited to:



The terms "heterocycle", "heterocyclyl", "heterocyclic radical", and "heterocyclic ring" are used interchangeably herein, and refer to a stable 3- to 8-membered monocyclic, a 7- to 12-membered bicyclic, or a 10- to 16-membered polycyclic heterocyclic moiety that is either saturated or partially unsaturated, and having, in addition to carbon atoms, one or more, such as one to four, heteroatoms, as defined above. When used in reference to a ring atom of a heterocycle, the term "nitrogen" includes a substituted nitrogen. As an example, in a saturated or partially unsaturated ring having 0–3 heteroatoms selected from oxygen, sulfur or nitrogen, the nitrogen may be N (as in 3,4-dihydro-2H-pyrrolyl), NH (as in pyrrolidinyl), or NR^+ (as in N-substituted pyrrolidinyl). A heterocyclic ring can be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure and any of the ring atoms can be optionally substituted. Examples of such saturated or partially unsaturated heterocyclic radicals include, without limitation, azetidinyl, oxetanyl, tetrahydrofuranyl, tetrahydrothienyl, pyrrolidinyl, piperidinyl, decahydroquinolinyl, oxazolidinyl, piperazinyl, tetrahydropyranyl, dioxanyl, dioxolanyl,

diazepinyl, oxazepinyl, thiazepinyl, morpholinyl, thiamorpholinyl, and . A heterocyclyl group may be mono-, bi-, tri-, or polycyclic, preferably mono-, bi-, or tricyclic, more preferably mono- or bicyclic. The term "heterocyclylalkyl" refers to an alkyl group substituted by a heterocyclyl, wherein the alkyl and heterocyclyl portions independently are optionally substituted. A bicyclic heterocyclic ring also includes groups in which the heterocyclic ring is fused to one or more aryl, heteroaryl, or cycloaliphatic rings. Exemplary bicyclic heterocyclic groups include indolinyl, isoindolinyl, benzodioxolyl, 1,3-dihydroisobenzofuranyl, 2,3-dihydrobenzofuranyl, and tetrahydroquinolinyl. A bicyclic heterocyclic ring can also be a spirocyclic ring system (e.g., 7- to 11-membered spirocyclic fused heterocyclic ring having, in addition to carbon atoms, one or more heteroatoms as defined above (e.g., one, two, three or four heteroatoms)). A bicyclic heterocyclic ring can also be a bridged ring system (e.g., 7- to 11-membered bridged heterocyclic ring having one, two, or three bridging atoms.

"Alkenyl" refers to an unsaturated branched or straight-chain alkyl group having the indicated number of carbon atoms (e.g., 2 to 8, or 2 to 6 carbon atoms) and at least one carbon-carbon double bond. The group may be in either the cis or trans configuration (Z or E configuration) about the double bond(s). Alkenyl groups include, but are not limited to, ethenyl, propenyl (e.g., prop-1-en-1-yl, prop-1-en-2-yl, prop-2-en-1-yl (allyl), prop-2-en-2-yl), and butenyl (e.g., but-1-en-1-yl, but-1-en-2-yl, 2-methyl-prop-1-en-1-yl, but-2-en-1-yl, but-2-en- 1-yl, but-2-en-2-yl, buta-1,3-dien-1-yl, buta-1,3-dien-2-yl).

"Alkynyl" refers to an unsaturated branched or straight-chain alkyl group having the indicated number of carbon atoms (e.g., 2 to 8 or 2 to 6 carbon atoms) and at least one carbon-carbon triple bond. Alkynyl groups include, but are not limited to, ethynyl, propynyl (e.g., prop-1-yn-1-yl, prop-2-yn-1-yl) and butynyl (e.g., but-1-yn-1-yl, but-1-yn-3-yl, but-3- yn-1-yl).

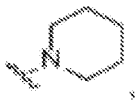
The term "oxo" is art-recognized and refers to a "=O" substituent. For example, a cyclopentane substituted with an oxo group is cyclopentanone.

The term "morpholinyl" refers to a substituent having the structure of:



which is optionally substituted.

The term "piperidinyl" refers to a substituent having a structure of:



which is optionally substituted.

In general, the term "substituted", whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at each position. Combinations of substituents envisioned under this invention are preferably those that result in the formation of stable or chemically feasible compounds. In some embodiments, "optionally substituted" is equivalent to "unsubstituted or substituted." In some embodiments, "optionally substituted" indicates that the designated atom or group is optionally substituted with one or more substituents independently selected from optional substituents provided herein. In some embodiments, optional substituent may be selected from the group consisting of: $C_{1\text{-}6}$alkyl, cyano, halogen, -O-$C_{1\text{-}6}$alkyl, $C_{1\text{-}6}$haloalkyl, $C_{3\text{-}7}$cycloalkyl, 3- to 7-membered heterocyclyl, 5-to 6-membered heteroaryl, and phenyl. In some embodiments, optional substituent is alkyl, cyano, halogen, halo, azide, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, carboxylic acid, -C(O)alkyl, -CO_2alkyl, carbonyl, carboxyl, alkylthio, sulfonyl, sulfonamido, sulfonamide, ketone, aldehyde, ester, heterocyclyl, aryl, or heteroaryl. In some embodiments, optional substituent is -OR^{s1}, -$NR^{s2}R^{s3}$, -$C(O)R^{s4}$, -$C(O)OR^{s5}$, $C(O)NR^{s6}R^{s7}$, -$OC(O)R^{s8}$, -$OC(O)OR^{s9}$, -$OC(O)NR^{s10}R^{s11}$, -$NR^{s12}C(O)R^{s13}$, or -$NR^{s14}C(O)OR^{s15}$, wherein R^{s1}, R^{s2}, R^{s3}, R^{s4}, R^{s5}, R^{s6}, R^{s7}, R^{s8}, R^{s9}, R^{s10}, R^{s11}, R^{s12}, R^{s13}, R^{s14}, and R^{s15} are each independently H, $C_{1\text{-}6}$ alkyl,

C_{3-10} cycloalkyl, C_{6-14} aryl, 5- to 10-membered heteroaryl, or 3- to 10-membered heterocyclyl, each of which is optionally substituted.

The term "haloalkyl" refers to an alkyl group that is substituted with at least one halogen. For example, $-CH_2F$, $-CHF_2$, $-CF_3$, $-CH_2CF_3$, $-CF_2CF_3$, and the like.

The term "cycloalkyl" refers to a monovalent saturated cyclic, bicyclic, bridged cyclic (e.g., adamantyl), or spirocyclic hydrocarbon group of 3-12, 3-10, 3-8, 4-8, or 4-6 carbons, referred to herein, e.g., as "C_{4-8}cycloalkyl," derived from a cycloalkane. In some embodiments, cycloalkyl is optionally substituted. Exemplary cycloalkyl groups include, but are not limited to, cyclohexanes, cyclopentanes, cyclobutanes and cyclopropanes. Unless specified otherwise, cycloalkyl groups are optionally substituted at one or more ring positions with, for example, alkanoyl, alkoxy, alkyl, haloalkyl, alkenyl, alkynyl, amido, amidino, amino, aryl, arylalkyl, azido, carbamate, carbonate, carboxy, cyano, cycloalkyl, ester, ether, formyl, halogen, haloalkyl, heteroaryl, heterocyclyl, hydroxyl, imino, ketone, nitro, phosphate, phosphonato, phosphinato, sulfate, sulfide, sulfonamido, sulfonyl or thiocarbonyl. In certain embodiments, the cycloalkyl group is not substituted, i.e., it is unsubstituted.

The terms "heterocyclyl" and "heterocyclic group" are art-recognized and refer to saturated, partially unsaturated, or aromatic 3- to 10-membered ring structures, alternatively 3-to 7-membered rings, whose ring structures include one to four heteroatoms, such as nitrogen, oxygen, and sulfur. In some embodiments, heterocyclyl is optionally substituted. The number of ring atoms in the heterocyclyl group can be specified using C_x-C_x nomenclature where x is an integer specifying the number of ring atoms. For example, a C_3-C_7heterocyclyl group refers to a saturated or partially unsaturated 3- to 7-membered ring structure containing one to four heteroatoms, such as nitrogen, oxygen, and sulfur. The designation "C_3-C_7" indicates that the heterocyclic ring contains a total of from 3 to 7 ring atoms, inclusive of any heteroatoms that occupy a ring atom position. One example of a C_3heterocyclyl is aziridinyl. Heterocycles may be, for example, mono-, bi-, or other multi-cyclic ring systems (e.g., fused, spiro, bridged bicyclic). A heterocycle may be fused to one or more aryl, partially unsaturated, or saturated rings. Heterocyclyl groups include, for example, biotinyl, chromenyl, dihydrofuryl, dihydroindolyl, dihydropyranyl, dihydrothienyl, dithiazolyl, homopiperidinyl, imidazolidinyl,

isoquinolyl, isothiazolidinyl, isooxazolidinyl, morpholinyl, oxolanyl, oxazolidinyl, phenoxanthenyl, piperazinyl, piperidinyl, pyranyl, pyrazolidinyl, pyrazolinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolidin-2-onyl, pyrrolinyl, tetrahydrofuryl, tetrahydroisoquinolyl, tetrahydropyranyl, tetrahydroquinolyl, thiazolidinyl, thiolanyl, thiomorpholinyl, thiopyranyl, xanthenyl, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. Unless specified otherwise, the heterocyclic ring is optionally substituted at one or more positions with substituents such as alkanoyl, alkoxy, alkyl, alkenyl, alkynyl, amido, amidino, amino, aryl, arylalkyl, azido, carbamate, carbonate, carboxy, cyano, cycloalkyl, ester, ether, formyl, halogen, haloalkyl, heteroaryl, heterocyclyl, hydroxyl, imino, ketone, nitro, oxo, phosphate, phosphonato, phosphinato, sulfate, sulfide, sulfonamido, sulfonyl and thiocarbonyl. In certain embodiments, the heterocyclyl group is not substituted, i.e., it is unsubstituted.

The term "aryl" is art-recognized and refers to a carbocyclic aromatic group. In some embodiments, aryl is optionally substituted. Representative aryl groups include phenyl, naphthyl, anthracenyl, and the like. The term "aryl" includes polycyclic ring systems having two or more carbocyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic and, e.g., the other ring(s) may be cycloalkyls, cycloalkenyls, cycloalkynyls, and/or aryls. Unless specified otherwise, the aromatic ring may be substituted at one or more ring positions with, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, carboxylic acid, -C(O)alkyl, CO_2alkyl, carbonyl, carboxyl, alkylthio, sulfonyl, sulfonamido, sulfonamide, ketone, aldehyde, ester, heterocyclyl, aryl or heteroaryl moieties, $-CF_3$, -CN, or the like. In certain embodiments, the aromatic ring is substituted at one or more ring positions with halogen, alkyl, hydroxyl, or alkoxyl. In certain other embodiments, the aromatic ring is not substituted, i.e., it is unsubstituted. In certain embodiments, the aryl group is a 6- to 10-membered ring structure. In some embodiments, the aryl group is a C_6-C_{14} aryl.

The term "heteroaryl" is art-recognized and refers to aromatic groups that include at least one ring heteroatom. In some embodiments, heteroaryl is optionally substituted. In certain instances, a heteroaryl group contains 1, 2, 3, or 4 ring heteroatoms. Representative examples of heteroaryl groups include pyrrolyl, furanyl, thiophenyl, imidazolyl, oxazolyl, thiazolyl, triazolyl, pyrazolyl, pyridinyl, pyrazinyl, pyridazinyl and pyrimidinyl, and the like. Unless specified otherwise, the

heteroaryl ring may be substituted at one or more ring positions with, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, carboxylic acid, C(O)alkyl, -CO_2alkyl, carbonyl, carboxyl, alkylthio, sulfonyl, sulfonamido, sulfonamide, ketone, aldehyde, ester, heterocyclyl, aryl or heteroaryl moieties, -CF_3, -CN, or the like. The term "heteroaryl" also includes polycyclic ring systems having two or more rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings may be cycloalkyls, cycloalkenyls, cycloalkynyls, and/or aryls. In certain embodiments, the heteroaryl ring is substituted at one or more ring positions with halogen, alkyl, hydroxyl, or alkoxyl. In certain other embodiments, the heteroaryl ring is not substituted, i.e., it is unsubstituted. In certain embodiments, the heteroaryl group is a 5- to 10-membered ring structure, alternatively a 5- to 6-membered ring structure, whose ring structure includes 1, 2, 3, or 4 heteroatoms, such as nitrogen, oxygen, and sulfur.

The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety represented by the general formula $-N(R^{10})(R^{11})$, wherein R^{10} and R^{11} each independently represent hydrogen, alkyl, cycloalkyl, heterocyclyl, alkenyl, aryl, aralkyl, or $(CH_2)_m$-R^{12}; or R^{10} and R^{11}, taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R^{12} represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocycle or a polycycle; and m is zero or an integer in the range of 1 to 8. In certain embodiments, R^{10} and R^{11} each independently represent hydrogen, alkyl, alkenyl, or -$(CH_2)_m$-R^{12}.

The terms "alkoxyl" or "alkoxy" are art-recognized and refer to an alkyl group, as defined above, having an oxygen radical attached thereto. In some embodiments, alkoxyl is optionally substituted. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like. An "ether" is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxyl, such as may be represented by one of -O-alkyl, -O-alkenyl, O-alkynyl, -O-$(CH_2)_m$-R^{12}, where m and R^{12} are described above. The term "haloalkoxyl" refers to an alkoxyl group that is substituted with at least one halogen. For example, -O-CH_2F, -O-CHF_2, -O-CF_3, and the like. In certain embodiments, the haloalkoxyl is an alkoxyl group that is substituted with at least one fluoro

group. In certain embodiments, the haloalkoxyl is an alkoxyl group that is substituted with from 1-6, 1-5, 1-4, 2-4, or 3 fluoro groups.

The symbol " ~~~ " indicates a point of attachment.

The compounds of the disclosure may contain one or more chiral centers and/or double bonds and, therefore, exist as stereoisomers, such as geometric isomers, enantiomers or diastereomers. The term "stereoisomers" when used herein consist of all geometric isomers, enantiomers or diastereomers. These compounds may be designated by the symbols "R" or "S," depending on the configuration of substituents around the stereogenic carbon atom. The present invention encompasses various stereoisomers of these compounds and mixtures thereof. Stereoisomers include enantiomers and diastereomers. Mixtures of enantiomers or diastereomers may be designated "(±)" in nomenclature, but the skilled artisan will recognize that a structure may denote a chiral center implicitly. It is understood that graphical depictions of chemical structures, e.g., generic chemical structures, encompass all stereoisomeric forms of the specified compounds, unless indicated otherwise.

Individual stereoisomers of compounds of the present invention can be prepared synthetically from commercially available starting materials that contain asymmetric or stereogenic centers, or by preparation of racemic mixtures followed by resolution methods well known to those of ordinary skill in the art. These methods of resolution are exemplified by (1) attachment of a mixture of enantiomers to a chiral auxiliary, separation of the resulting mixture of diastereomers by recrystallization or chromatography and liberation of the optically pure product from the auxiliary, (2) salt formation employing an optically active resolving agent, or (3) direct separation of the mixture of optical enantiomers on chiral chromatographic columns. Stereoisomeric mixtures can also be resolved into their component stereoisomers by well-known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Further, enantiomers can be separated using supercritical fluid chromatographic (SFC) techniques described in the literature. Still further, stereoisomers can be obtained from stereomerically-pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

Geometric isomers can also exist in the compounds of the present invention. The present invention encompasses the various geometric isomers and mixtures thereof resulting from the arrangement of substituents around a carbon-carbon double bond or arrangement of substituents around a carbocyclic ring. Substituents around a carbon-carbon double bond are designated as being in the "Z" or "E" configuration wherein the terms "Z" and "E" are used in accordance with IUPAC standards. Unless otherwise specified, structures depicting double bonds encompass both the "E" and "Z" isomers.

Substituents around a carbon-carbon double bond alternatively can be referred to as "cis" or "trans," where "cis" represents substituents on the same side of the double bond and "trans" represents substituents on opposite sides of the double bond. The arrangement of substituents around a carbocyclic ring are designated as "cis" or "trans." The term "cis" represents substituents on the same side of the plane of the ring and the term "trans" represents substituents on opposite sides of the plane of the ring. Mixtures of compounds wherein the substituents are disposed on both the same and opposite sides of plane of the ring are designated "cis/trans."

The invention also embraces isotopically labeled compounds of the invention which are identical to those recited herein, except that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl, respectively.

Certain isotopically-labeled disclosed compounds (e.g., those labeled with ^{3}H and ^{14}C) are useful in compound and/or substrate tissue distribution assays. Tritiated (i.e., ^{3}H) and carbon-14 (i.e., ^{14}C) isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (i.e., ^{2}H) may afford certain therapeutic advantages resulting from greater metabolic stability (e.g., increased in vivo half-life or reduced dosage requirements) and hence may be preferred in some circumstances. Isotopically labeled compounds of the invention can generally be prepared by following procedures analogous to those disclosed in, e.g., the Examples herein by substituting an isotopically labeled reagent for a non-isotopically labeled reagent.

As used herein, the terms "subject" and "patient" refer to organisms to be treated by the methods of the present invention. Such organisms are preferably mammals (e.g., murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably humans.

As used herein, the term "pharmaceutical composition" refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*.

As used herein, the term "pharmaceutically acceptable excipient" refers to any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (e.g., such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro; Lippincott, Williams & Wilkins, Baltimore, MD, 2006.

As is known to those of skill in the art, "salts" of the compounds of the present invention may be derived from inorganic or organic acids and bases. Examples of acids include, but are not limited to, hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic, benzenesulfonic acid, and the like. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

Examples of bases include, but are not limited to, alkali metal (e.g., sodium) hydroxides, alkaline earth metal (e.g., magnesium) hydroxides, ammonia, and compounds of formula NW_4^+, wherein W is C_{1-4} alkyl, and the like.

Examples of salts include, but are not limited to: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, flucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-

naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate, persulfate, phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, undecanoate, and the like. Other examples of salts include anions of the compounds of the present invention compounded with a suitable cation such as Na^+, NH_4^+, and NW_4^+ (wherein W is a C_{1-4} alkyl group), and the like.

Abbreviations as used herein include diisopropylethylamine (DIPEA); 4-dimethylaminopyridine (DMAP); tetrabutylammonium iodide (TBAI); 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC); benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), 9-Fluorenylmethoxycarbonyl (Fmoc), tetrabutyldimethylsilyl chloride (TBDMSCl), hydrogen fluoride (HF), phenyl (Ph), bis(trimethylsilyl)amine (HMDS), dimethylformamide (DMF); methylene chloride (DCM); tetrahydrofuran (THF); high-performance liquid chromatography (HPLC); mass spectrometry (MS), evaporative light scattering detector (ELSD), electrospray (ES)); nuclear magnetic resonance spectroscopy (NMR).

As used herein, the term "effective amount" refers to the amount of a compound (e.g., a nucleic acid, e.g., an mRNA) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route. The term effective amount can be considered to include therapeutically and/or prophylactically effective amounts of a compound.

The phrase "therapeutically effective amount" as used herein means that amount of a compound (e.g., a nucleic acid, e.g., an mRNA), material, or composition comprising a compound (e.g., a nucleic acid, e.g., an mRNA) which is effective for producing some desired therapeutic effect in at least a sub-population of cells in a mammal, for example, a human, or a subject (e.g., a human subject) at a reasonable benefit/risk ratio applicable to any medical treatment.

The phrase "prophylactically effective amount" as used herein means that amount of a compound (e.g., a nucleic acid, e.g., an mRNA), material, or composition comprising a compound (e.g., a nucleic acid, e.g., an mRNA) which is effective for producing some desired prophylactic effect in at least a sub-population of cells in a mammal, for example, a human, or a subject (e.g., a human subject) by reducing, minimizing or eliminating the risk of developing a condition or the

reducing or minimizing severity of a condition at a reasonable benefit/risk ratio applicable to any medical treatment.

As used herein, the terms "treat," "treating," and "treatment" include any effect, e.g., lessening, reducing, modulating, ameliorating or eliminating, that results in the improvement of the condition, disease, disorder, and the like, or ameliorating a symptom thereof.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it should be understood that the element or component can be any one of the recited elements or components, or the element or component can be selected from a group consisting of two or more of the recited elements or components.

Further, it should be understood that elements and/or features of a composition or a method described herein can be combined in a variety of ways without departing from the spirit and scope of the present invention, whether explicit or implicit herein. For example, where reference is made to a particular compound, that compound can be used in various embodiments of compositions of the present invention and/or in methods of the present invention, unless otherwise understood from the context. In other words, within this application, embodiments have been described and depicted in a way that enables a clear and concise application to be written and drawn, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the present teachings and invention(s). For example, it will be appreciated that all features described and depicted herein can be applicable to all aspects of the invention(s) described and depicted herein.

It should be understood that the expression "at least one of" includes individually each of the recited objects after the expression and the various combinations of two or more of the recited objects unless otherwise understood from the context and use. The expression "and/or" in

connection with three or more recited objects should be understood to have the same meaning unless otherwise understood from the context.

The use of the term "include," "includes," "including," "have," "has," "having," "contain," "contains," or "containing," including grammatical equivalents thereof, should be understood generally as open-ended and non-limiting, for example, not excluding additional unrecited elements or steps, unless otherwise specifically stated or understood from the context.

Where the use of the term "about" is before a quantitative value, the present invention also includes the specific quantitative value itself, unless specifically stated otherwise. As used herein, the term "about" refers to a ±10% variation from the nominal value unless otherwise indicated or inferred.

As used herein, unless otherwise indicated, the term "antibody" means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen. It is understood the term encompasses an intact antibody (e.g., an intact monoclonal antibody), or a fragment thereof, such as an Fc fragment of an antibody (e.g., an Fc fragment of a monoclonal antibody), or an antigen-binding fragment of an antibody (e.g., an antigen-binding fragment of a monoclonal antibody), including an intact antibody, antigen-binding fragment, or Fc fragment that has been modified or engineered. Examples of antigen-binding fragments include Fab, Fab', $(Fab')_2$, Fv, single chain antibodies (e.g., scFv), minibodies, and diabodies. Examples of antibodies that have been modified or engineered include chimeric antibodies, humanized antibodies, and multispecific antibodies (e.g., bispecific antibodies). The term also encompasses an immunoglobulin single variable domain, such as a Nanobody (e.g., a V_{HH}).

As used here, an "antibody that binds to X" (i.e., X being a particular antigen), or "an anti-X antibody", is an antibody that specifically recognizes the antigen X.

As used herein, a "buried interchain disulfide bond" or an "interchain buried disulfide bond" refers to a disulfide bond on a polypeptide which is not readily accessible to water soluble reducing agents, or is effectively "buried" in the hydrophobic regions of the polypeptide, such that it is unavailable to both reducing agents and for conjugation to other hydrophilic PEGs.

Buried interchain disulfide bonds are further described in WO2017096361A1, which is incorporated by reference in its entirety.

As used herein, specificity of the targeted delivery by an LNP is defined by the ratio between % of a desired immune cell type that receives the delivered nucleic acid (e.g., on-target delivery), and % of an undesired immune cell type that is not meant to be the destination of the delivery, but receives the delivered nucleic acid (e.g., off-target delivery). For example, the specificity is higher when more desired immune cells receive the delivered nucleic acid, while less undesired immune cells receive the delivered nucleic acid. Specificity of the targeted delivery by an LNP can also be defined the ratio of amount of nucleic acid being delivered to the desired immune cells (e.g., on-target delivery) and amount of nucleic acid being delivered to the undesired immune cells (e.g., off-target delivery). Specificity of the delivery can be determined using any suitable method. As a non-limiting example, expression level of the nucleic acid in the desired immune cell type can be measured and compared to that of a different immune cell type that is not meant to be the destination of the delivery.

As used herein, in some embodiments, a reference LNP is an LNP that does not have the immune cell targeting group but is otherwise the same as the tested LNP. In some other embodiments, a reference LNP is an LNP that has a different ionizable cationic lipid but is otherwise the same as the tested LNP. In some embodiments, a reference LNP comprises ALC-0315 as the ionizable cationic lipid which is different from the ionizable cationic lipid in a tested LNP, but is otherwise the same as the tested LNP.

As used herein, a humanized antibody is an antibody which is wholly or partially of non-human origin and whose protein sequence has been modified to replace certain amino acids, for instance that occur at the corresponding position(s) in the framework regions of the VH and VL domains in a sequence of antibody from a human being, to increase its similarity to antibodies produced naturally in humans, in order to avoid or minimize an immune response in humans. For example, using techniques of genetic engineering, the variable domains of a non-human antibodies of interest may be combined with the constant domains of human antibodies. The constant domains of a humanized antibody are most of the time human CH and CL domains.

As used herein, the term "structural lipid" refers to sterols and also to lipids containing sterol moieties.

It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present invention remain operable. Moreover, two or more steps or actions may be conducted simultaneously.

At various places in the present specification, substituents are disclosed in groups or in ranges. It is specifically intended that the description include each and every individual subcombination of the members of such groups and ranges. For example, the term "C_{1-6} alkyl" is specifically intended to individually disclose C_1, C_2, C_3, C_4, C_5, C_6, C_1-C_6, C_1-C_5, C_1-C_4, C_1-C_3, C_1-C_2, C_2-C_6, C_2-C_5, C_2-C_4, C_2-C_3, C_3-C_6, C_3-C_5, C_3-C_4, C_4-C_6, C_4-C_5, and C_5-C_6 alkyl. By way of other examples, an integer in the range of 0 to 40 is specifically intended to individually disclose 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, and 40, and an integer in the range of 1 to 20 is specifically intended to individually disclose 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20.

The use of any and all examples, or exemplary language herein, for example, "such as" or "including," is intended merely to illustrate better the present invention and does not pose a limitation on the scope of the invention unless claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the present invention.

Throughout the description, where compositions and kits are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions and kits of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

As a general matter, compositions specifying a percentage are by weight unless otherwise specified. Further, if a variable is not accompanied by a definition, then the previous definition of the variable controls.

Immunoglobulin single variable domain

In some embodiments, the immune cell targeting group of the LNPs as described herein comprise an immunoglobulin single variable domain, such as a Nanobody.

The term "immunoglobulin single variable domain" ("ISV"), interchangeably used with "single variable domain," defines immunoglobulin molecules wherein the antigen binding site is present on, and formed by, a single immunoglobulin domain. This sets immunoglobulin single variable domains apart from "conventional" immunoglobulins (e.g., monoclonal antibodies) or their fragments (such as Fab, Fab', $F(ab')_2$, scFv, di-scFv), wherein two immunoglobulin domains, in particular two variable domains, interact to form an antigen binding site. Typically, in conventional immunoglobulins, a heavy chain variable domain (V_H) and a light chain variable domain (V_L) interact to form an antigen binding site. In this case, the complementarity determining regions (CDRs) of both V_H and V_L will contribute to the antigen binding site, i.e. a total of 6 CDRs will be involved in antigen binding site formation. In view of the above definition, the antigen-binding domain of a conventional 4-chain antibody (such as an IgG, IgM, IgA, IgD or IgE molecule; known in the art) or of a Fab, a $F(ab')_2$ fragment, an Fv fragment such as a disulfide linked Fv or a scFv fragment, or a diabody (all known in the art) derived from such conventional 4-chain antibody, would normally not be regarded as an immunoglobulin single variable domain, as, in these cases, binding to the respective epitope of an antigen would normally not occur by one (single) immunoglobulin domain but by a pair of (associating) immunoglobulin domains such as light and heavy chain variable domains, i.e., by a V_H-V_L pair of immunoglobulin domains, which jointly bind to an epitope of the respective antigen.

In contrast, immunoglobulin single variable domains are capable of specifically binding to an epitope of the antigen without pairing with an additional immunoglobulin variable domain. The binding site of an immunoglobulin single variable domain is formed by a single V_H, a single V_{HH} or single V_L domain. Hence, the antigen binding site of an immunoglobulin single variable domain is formed by no more than three CDRs.

As such, the single variable domain may be a light chain variable domain sequence (e.g., a V_L-sequence) or a suitable fragment thereof; or a heavy chain variable domain sequence (e.g., a V_H-sequence or V_{HH} sequence) or a suitable fragment thereof; as long as it is capable of forming a single antigen binding unit (i.e., a functional antigen binding unit that essentially consists of the single variable domain, such that the single antigen binding domain does not need to interact with another variable domain to form a functional antigen binding unit).

An immunoglobulin single variable domain (ISV) can for example be a heavy chain ISV, such as a V_H, V_{HH}, including a camelized V_H or humanized V_{HH}. In one embodiment, it is a V_{HH}, including a camelized V_H or humanized V_{HH}. Heavy chain ISVs can be derived from a conventional four-chain antibody or from a heavy chain antibody.

For example, the immunoglobulin single variable domain may be a (single) domain antibody (or an amino acid sequence that is suitable for use as a single domain antibody), a "dAb" or dAb (or an amino acid sequence that is suitable for use as a dAb) or a Nanobody® ISV (as defined herein and including but not limited to a V_{HH}); other single variable domains, or any suitable fragment of any one thereof.

In particular, the immunoglobulin single variable domain may be a Nanobody® ISV (such as a V_{HH}, including a humanized V_{HH} or camelized V_H) or a suitable fragment thereof. [Note: Nanobody® is a registered trademark of Ablynx N.V.].

"V_{HH} domains", also known as V_{HH}s, V_{HH} antibody fragments, and V_{HH} antibodies, have originally been described as the antigen binding immunoglobulin variable domain of "heavy chain antibodies" (i.e., of "antibodies devoid of light chains"; Hamers-Casterman et al.1993 (Nature 363: 446-448). The term "V_{HH} domain" has been chosen in order to distinguish these variable domains from the heavy chain variable domains that are present in conventional 4-chain antibodies (which are referred to herein as "V_H domains") and from the light chain variable domains that are present in conventional 4-chain antibodies (which are referred to herein as "V_L domains"). For a further description of V_{HH}'s, reference is made to the review article by Muyldermans 2001 (Reviews in Molecular Biotechnology 74: 277-302).

For the term "dAb's" and "domain antibody", reference is for example made to Ward et al.1989 (Nature 341: 544), to Holt et al.2003 (Trends Biotechnol.21: 484); as well as to for example WO 2004/068820, WO 2006/030220, WO 2006/003388 and other published patent applications of Domantis Ltd. It should also be noted that, although less preferred in the context of the present invention because they are not of mammalian origin, single variable domains can be derived from certain species of shark (for example, the so-called "IgNAR domains", see for example WO 2005/18629).

Typically, the generation of immunoglobulins involves the immunization of experimental animals, fusion of immunoglobulin producing cells to create hybridomas and screening for the desired specificities. Alternatively, immunoglobulins can be generated by screening of naïve, immune or synthetic libraries, e.g., by phage display.

The generation of immunoglobulin sequences, such as V_{HH}s, has been described extensively in various publications, among which WO 1994/04678, Hamers-Casterman et al. 1993 (Nature 363: 446-448) and Muyldermans et al. 2001 (Reviews in Molecular Biotechnology 74: 277-302, 2001). In these methods, camelids are immunized with the target antigen in order to induce an immune response against said target antigen. The repertoire of V_{HH}s obtained from said immunization is further screened for V_{HH}s that bind the target antigen.

In these instances, the generation of antibodies requires purified antigen for immunization and/or screening. Antigens can be purified from natural sources, or in the course of recombinant production. Immunization and/or screening for immunoglobulin sequences can be performed using peptide fragments of such antigens.

Immunoglobulin sequences of different origin, comprising mouse, rat, rabbit, donkey, human and camelid immunoglobulin sequences can be used herein. Also, fully human, humanized or chimeric sequences can be used in the method described herein. For example, camelid immunoglobulin sequences and humanized camelid immunoglobulin sequences, or camelized domain antibodies, e.g., camelized dAb as described by Ward et al. 1989 (Nature 341: 544), WO 1994/04678, and Davis and Riechmann (1994, Febs Lett., 339:285-290; and 1996, Prot. Eng., 9:531-537) can be used herein. Moreover, the ISVs are fused forming a multivalent and/or multispecific construct (for multivalent and multispecific polypeptides containing one or more

V_{HH} domains and their preparation, reference is also made to Conrath et al.2001 (J. Biol. Chem., Vol.276, 10.7346-7350) as well as to for example WO 1996/34103 and WO 1999/23221).

A "humanized V_{HH}" comprises an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring V_{HH} domain, but that has been "humanized", i.e., by replacing one or more amino acid residues in the amino acid sequence of said naturally occurring V_{HH} sequence (and in particular in the framework sequences) by one or more of the amino acid residues that occur at the corresponding position(s) in a V_H domain from a conventional 4-chain antibody from a human being (e.g., indicated above). This can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the prior art (e.g., WO 2008/020079). Again, it should be noted that such humanized V_{HH}s can be obtained in any suitable manner known per se and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring VHH domain as a starting material.

A "camelized V_H" comprises an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring V_H domain, but that has been "camelized", i.e. by replacing one or more amino acid residues in the amino acid sequence of a naturally occurring V_H domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a V_{HH} domain of a (camelid) heavy chain antibody. This can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the description in the prior art (e.g., Davies and Riechman 1994, FEBS 339: 285; 1995, Biotechnol.13: 475; 1996, Prot. Eng.9: 531; and Riechman 1999, J. Immunol. Methods 231: 25). Such "camelizing" substitutions are inserted at amino acid positions that form and/or are present at the V_H-V_L interface, and/or at the so-called Camelidae hallmark residues, as defined herein (see for example WO 1994/04678 and Davies and Riechmann (1994 and 1996, supra). In one embodiment, the V_H sequence that is used as a starting material or starting point for generating or designing the camelized V_H is a V_H sequence from a mammal, such as the V_H sequence of a human being, such as a V_H3 sequence. However, it should be noted that such camelized V_H can be obtained in any suitable manner known per se and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring V_H domain as a starting material.

The structure of an immunoglobulin single variable domain sequence can be considered to be comprised of four framework regions ("FRs"), which are referred to in the art and herein as "Framework region 1" ("FR1"); as "Framework region 2" ("FR2"); as "Framework region 3" ("FR3"); and as "Framework region 4" ("FR4"), respectively; which framework regions are interrupted by three complementary determining regions ("CDRs"), which are referred to in the art and herein as "Complementarity Determining Region 1" ("CDR1"); as "Complementarity Determining Region 2" ("CDR2"); and as "Complementarity Determining Region 3" ("CDR3"), respectively.

In such an immunoglobulin sequence, the framework sequences may be any suitable framework sequences, and examples of suitable framework sequences will be clear to the skilled person, for example on the basis the standard handbooks and the further disclosure and prior art mentioned herein.

The framework sequences are (a suitable combination of) immunoglobulin framework sequences or framework sequences that have been derived from immunoglobulin framework sequences (for example, by humanization or camelization). For example, the framework sequences may be framework sequences derived from a light chain variable domain (e.g., a V_L-sequence) and/or from a heavy chain variable domain (e.g., a V_H-sequence or V_{HH} sequence). In one particular aspect, the framework sequences are either framework sequences that have been derived from a V_{HH}-sequence (in which said framework sequences may optionally have been partially or fully humanized) or are conventional V_H sequences that have been camelized (as defined herein).

In particular, the framework sequences present in the ISV sequence described herein may contain one or more of hallmark residues (as defined herein), such that the ISV sequence is a Nanobody® ISV, such as, e.g., a V_{HH}, including a humanized V_{HH} or camelized V_H. Non-limiting examples of (suitable combinations of) such framework sequences will become clear from the further disclosure herein.

The total number of amino acid residues in a V_H domain and a V_{HH} domain will usually be in the range of from 110 to 120, often between 112 and 115. It should however be noted that smaller and longer sequences may also be suitable for the purposes described herein.

However, it should be noted that the ISVs described herein is not limited as to the origin of the ISV sequence (or of the nucleotide sequence used to express it), nor as to the way that the ISV sequence or nucleotide sequence is (or has been) generated or obtained. Thus, the ISV sequences may be naturally occurring sequences (from any suitable species) or synthetic or semi-synthetic sequences. In a specific but non-limiting aspect, the ISV sequence is a naturally occurring sequence (from any suitable species) or a synthetic or semi-synthetic sequence, including but not limited to "humanized" (as defined herein) immunoglobulin sequences (such as partially or fully humanized mouse or rabbit immunoglobulin sequences, and in particular partially or fully humanized V_{HH} sequences), "camelized" (as defined herein) immunoglobulin sequences (and in particular camelized V_H sequences), as well as ISVs that have been obtained by techniques such as affinity maturation (for example, starting from synthetic, random or naturally occurring immunoglobulin sequences), CDR grafting, veneering, combining fragments derived from different immunoglobulin sequences, PCR assembly using overlapping primers, and similar techniques for engineering immunoglobulin sequences well known to the skilled person; or any suitable combination of any of the foregoing.

Similarly, nucleotide sequences may be naturally occurring nucleotide sequences or synthetic or semi-synthetic sequences, and may for example be sequences that are isolated by PCR from a suitable naturally occurring template (e.g., DNA or RNA isolated from a cell), nucleotide sequences that have been isolated from a library (and in particular, an expression library), nucleotide sequences that have been prepared by introducing mutations into a naturally occurring nucleotide sequence (using any suitable technique known per se, such as mismatch PCR), nucleotide sequence that have been prepared by PCR using overlapping primers, or nucleotide sequences that have been prepared using techniques for DNA synthesis known per se.

Generally, Nanobody® ISVs (in particular V_{HH} sequences, including (partially) humanized V_{HH} sequences and camelized V_H sequences) can be characterized by the presence of one or more "Hallmark residues" (as described herein) in one or more of the framework sequences (again as further described herein). Thus, generally, a Nanobody® ISV can be defined as an immunoglobulin sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which one or more of the Hallmark residues are as further defined herein.

In particular, a Nanobody® ISV can be an immunoglobulin sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which the framework sequences are as further defined herein.

More in particular, a Nanobody® ISV can be an immunoglobulin sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which: one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are Hallmark residues.

In one embodiment, the immunoglobulin single variable domain has certain amino acid substitutions in the framework regions effective in preventing or reducing binding of so-called "pre-existing antibodies" to the polypeptides. ISVs in which (i) the amino acid residue at position 112 is one of K or Q; and/or (ii) the amino acid residue at position 89 is T; and/or (iii) the amino acid residue at position 89 is L and the amino acid residue at position 110 is one of K or Q; and (iv) in each of cases (i) to (iii), the amino acid at position 11 is preferably V have been described in WO2015/173325.

Polypeptides

The immunoglobulin single variable domains may form part of a protein or polypeptide, which may comprise or essentially consist of one or more (at least one) immunoglobulin single variable domains and which may optionally further comprise one or more further amino acid sequences

(all optionally linked via one or more suitable linkers). The term "immunoglobulin single variable domain" may also encompass such polypeptides. The one or more immunoglobulin single variable domains may be used as a binding unit in such a protein or polypeptide, which may optionally contain one or more further amino acids that can serve as a binding unit, so as to provide a monovalent, multivalent or multispecific polypeptide of the invention, respectively (for multivalent and multispecific polypeptides containing one or more VHH domains and their preparation, reference is also made to Conrath et al. 2001 (J. Biol. Chem.276: 7346), as well as to for example WO 1996/34103, WO 1999/23221 and WO 2010/115998).

The polypeptides may comprise or essentially consist of one immunoglobulin single variable domain, as outlined above. Such polypeptides are also referred to herein as monovalent polypeptides.

The term "multivalent" indicates the presence of multiple ISVs in a polypeptide. In one embodiment, the polypeptide is "bivalent", i.e., comprises or consists of two ISVs. In one embodiment, the polypeptide is "trivalent", i.e., comprises or consists of three ISVs. In another embodiment, the polypeptide is "tetravalent", i.e. comprises or consists of four ISVDs. The polypeptide can thus be "bivalent", "trivalent", "tetravalent", "pentavalent", "hexavalent", "heptavalent", "octavalent", "nonavalent", etc., i.e., the polypeptide comprises or consists of two, three, four, five, six, seven, eight, nine, etc., ISVs, respectively. In one embodiment the multivalent ISV polypeptide is trivalent. In another embodiment the multivalent ISV polypeptide is tetravalent. In still another embodiment, the multivalent ISV polypeptide is pentavalent.

In one embodiment, the multivalent ISV polypeptide can also be multispecific. The term "multispecific" refers to binding to multiple different target molecules (also referred to as antigens). The multivalent ISV polypeptide can thus be "bispecific", "trispecific", "tetraspecific", etc., i.e., can bind to two, three, four, etc., different target molecules, respectively.

For example, the polypeptide may be bispecific-trivalent, such as a polypeptide comprising or consisting of three ISVs, wherein two ISVs bind to a first target and one ISV binds to a second target different from the first target. In another example, the polypeptide may be trispecific-tetravalent, such as a polypeptide comprising or consisting of four ISVs, wherein one ISV binds

to a first target, two ISVs bind to a second target different from the first target and one ISV binds to a third target different from the first and the second target. In still another example, the polypeptide may be trispecific-pentavalent, such as a polypeptide comprising or consisting of five ISVs, wherein two ISVs bind to a first target, two ISVs bind to a second target different from the first target and one ISV binds to a third target different from the first and the second target.

In one embodiment, the multivalent ISV polypeptide can also be multiparatopic. The term "multiparatopic" refers to binding to multiple different epitopes on the same target molecules (also referred to as antigens). The multivalent ISV polypeptide can thus be "biparatopic", "triparatopic", etc., i.e., can bind to two, three, etc., different epitopes on the same target molecules, respectively.

In another aspect, a polypeptide described herein that comprises or essentially consists of one or more immunoglobulin single variable domains (or suitable fragments thereof), may further comprise one or more other groups, residues, moieties or binding units. Such further groups, residues, moieties, binding units or amino acid sequences may or may not provide further functionality to the immunoglobulin single variable domain (and/or to the polypeptide in which it is present) and may or may not modify the properties of the immunoglobulin single variable domain.

For example, such further groups, residues, moieties or binding units may be one or more additional amino acids, such that the compound, construct or polypeptide is a (fusion) protein or (fusion) polypeptide. In a preferred but non-limiting aspect, said one or more other groups, residues, moieties or binding units are immunoglobulins. Even more preferably, said one or more other groups, residues, moieties or binding units are chosen from the group consisting of domain antibodies, amino acids that are suitable for use as a domain antibody, single domain antibodies, amino acids that are suitable for use as a single domain antibody, "dAb"s, amino acids that are suitable for use as a dAb, or Nanobodies.

Alternatively, such groups, residues, moieties or binding units may for example be chemical groups, residues, moieties, which may or may not by themselves be biologically and/or pharmacologically active. For example, and without limitation, such groups may be linked to the

one or more immunoglobulin single variable domain so as to provide a "derivative" of the immunoglobulin single variable domain.

In another embodiment, said further residues may be effective in preventing or reducing binding of so-called "pre-existing antibodies" to the polypeptides. For this purpose, the polypeptides and constructs may contain a C-terminal extension (X)n (in which n is 1 to 10, preferably 1 to 5, such as 1, 2, 3, 4 or 5 (and preferably 1 or 2, such as 1); and each X is an (preferably naturally occurring) amino acid residue that is independently chosen, and preferably independently chosen from the group consisting of alanine (A), glycine (G), valine (V), leucine (L) or isoleucine (I), for which reference is made to WO 2012/175741. Accordingly, the polypeptide may further comprise a C-terminal extension (X)n, in which n is 1 to 5, such as 1, 2, 3, 4 or 5, and in which X is a naturally occurring amino acid, preferably no cysteine.

In the polypeptides described above, the one or more immunoglobulin single variable domains and the one or more groups, residues, moieties or binding units may be linked directly to each other and/or via one or more suitable linkers or spacers. For example, when the one or more groups, residues, moieties or binding units are amino acids, the linkers may also be an amino acid, so that the resulting polypeptide is a fusion protein or fusion polypeptide.

As used herein, the term "linker" denotes a peptide that fuses together two or more ISVs into a single molecule. The use of linkers to connect two or more (poly)peptides is well known in the art. Further exemplary peptidic linkers are shown in Table B. One often used class of peptidic linker are known as the "Gly-Ser" or "GS" linkers. These are linkers that essentially consist of glycine (G) and serine (S) residues, and usually comprise one or more repeats of a peptide motif such as the GGGGS (SEQ ID NO:154) motif (for example, having the formula (Gly-Gly-Gly-Gly-Ser)n (SEQ ID NO: 152) in which n may be 1, 2, 3, 4, 5, 6, 7 or more). Some often-used examples of such GS linkers are 9GS linkers (GGGGSGGGS, SEQ ID NO: 157), 15GS linkers (n=3) (SEQ ID NO: 158) and 35GS linkers (n=7) (SEQ ID NO: 159). Reference is for example made to Chen et al. 2013 (Adv. Drug Deliv. Rev. 65(10): 1357–1369) and Klein et al. 2014 (Protein Eng. Des. Sel.27 (10): 325-330).

Generally, it should be noted that the term Nanobody as used herein in its broadest sense is not limited to a specific biological source or to a specific method of preparation. For example, as will be discussed in more detail below, the Nanobodies can be obtained (1) by isolating the VHH domain of a naturally occurring heavy chain antibody; (2) by expression of a nucleotide sequence encoding a naturally occurring VHH domain; (3) by "humanization" (as described below) of a naturally occurring VHH domain or by expression of a nucleic acid encoding a such humanized VHH domain; (4) by "camelization" (as described below) of a naturally occurring VH domain from any animal species, in particular a species of mammal, such as from a human being, or by expression of a nucleic acid encoding such a camelized VH domain; (5) by "camelisation" of a "domain antibody" or "Dab" as described by Ward et al (supra), or by expression of a nucleic acid encoding such a camelized VH domain; (6) using synthetic or semi-synthetic techniques for preparing proteins, polypeptides or other amino acid sequences; (7) by preparing a nucleic acid encoding a Nanobody using techniques for nucleic acid synthesis, followed by expression of the nucleic acid thus obtained; and/or (8) by any combination of the foregoing. Suitable methods and techniques for performing the foregoing will be clear to the skilled person based on the disclosure herein and for example include the methods and techniques described in more detail hereinbelow.

II. IONIZABLE CATIONIC LIPIDS

Provided herein are ionizable cationic lipids that can be used to produce lipid nanoparticle compositions to facilitate the delivery of a payload (e.g., a nucleic acid, such as a DNA or RNA, such as an mRNA) disposed therein to cells, e.g., mammalian cells, e.g., immune cells. The ionizable cationic lipids have been designed to enable intracellular delivery of a nucleic acid, e.g., mRNA, to the cytosolic compartment of a target cell type and rapidly degrade into non-toxic components. The complex functionalities of the ionizable cationic lipids are facilitated by the interplay between the chemistry and geometry of the ionizable lipid head group, the hydrophobic "acyl-tail" groups and the linkers connecting the head group and the acyl tail groups. Typically, the pK_a of the ionizable amine head group is designed to be in the range of 6-8, such as between 6.2-7.4, or between 6.7-7.2, such that it remains strongly cationic under acidic formulation conditions (e.g., pH 4 – pH 5.5), neutral or slightly anionic in physiological pH (7.4)

and cationic in the early and late endosomal compartments (e.g., pH 5.5 – pH 7). The acyl-tail groups play a key role in fusion of the lipid nanoparticle with endosomal membranes and membrane destabilization through structural perturbation. The three-dimensional structure of the acyl-tail (determined by its length, and degree and site of unsaturation) along with the relative sizes of the head group and tail group are thought to play a role in promoting membrane fusion, and hence lipid nanoparticle endosomal escape (a key requirement for cytosolic delivery of a nucleic acid payload). The linker connecting the head group and acyl tail groups is designed to degrade by physiologically prevalent enzymes (e.g., esterases, or proteases) or by acid catalyzed hydrolysis.

In some embodiments, the ionizable cationic lipid is present in the lipid blend in a range of 40-60 mole percent.

III. LIPID-IMMUNE CELL TARGETING GROUP CONJUGATES

In one aspect, provided herein are lipid nanoparticles (LNPs) comprising a lipid blend for targeted delivery of a nucleic acid into a cell, e.g., an immune cell. The LNPs may comprise a novel cationic lipid as described herein. In some embodiments, the lipid blend comprises a lipid-immune cell targeting group conjugate comprising the compound of Formula (II): [Lipid] – [optional linker] – [immune cell targeting group]. In some embodiments, the lipid blend comprises an ionizable cationic lipid. In some embodiments, the ionizable cationic lipid comprises an ionizable cationic lipid as described herein. In some embodiments, the LNP comprises a nucleic acid disposed therein.

In some embodiments, the immune cell targeting group comprises an antibody that binds a T cell antigen. In some embodiments, the T cell antigen is CD3, CD4, CD7, or CD8, or a combination thereof (e.g., both CD3 and CD8, both CD4 and CD8, or both CD7 and CD8). In some embodiments, the immune cell targeting group comprises an antibody that binds a Natural Killer (NK) cell antigen. In some embodiments, the NK cell antigen is CD7, CD8, or CD56, or a combination thereof (e.g., both CD7 and CD8). In some embodiments, the antibody is a human or humanized antibody.

In some embodiments, the immune cell targeting group comprise a single antibody that binds to CD3 or CD7.

In some embodiments, the immune cell targeting group is covalently coupled to a lipid in the lipid blend via a polyethylene glycol (PEG) containing linker. In some embodiments, the lipid covalently coupled to the immune cell targeting group via a PEG containing linker is distearoylglycerol (DSG), distearoyl-phosphatidylethanolamine (DSPE), dimyrstoyl-phosphatidylethanolamine (DMPE), distearoyl-glycero-phosphoglycerol (DSPG), dimyristoyl-glycerol (DMG), dipalmitoyl-phosphatidylethanolamine (DPPE), dipalmitoyl-glycerol (DPG), dialkylacetamide, or ceramide. In some embodiments, the PEG is PEG 2000. In some embodiments, the PEG is PEG 3400.

In some embodiments, the lipid-immune cell targeting group conjugate is present in the lipid blend in a range of 0.002-0.2 mole percent.

In some embodiments, the immune cell targeting group comprises an antibody, and the antibody is a Fab or an immunoglobulin single variable domain, such as a Nanobody. In some embodiments, the immune cell targeting group comprises a Fab, F(ab')2, Fab'-SH, Fv, or scFv fragment. In some embodiments, the immune cell targeting group comprises a Fab that is engineered to knock out one or more natural interchain disulfide bonds. For example, in some embodiments, the Fab comprises a heavy chain fragment that comprises C233S substitution, numbering according to Kabat, and/or a light chain fragment that comprises C214S substitution, numbering according to Kabat. In some embodiments, the Fab comprises a non-natural interchain disulfide. In some embodiments, the immune cell targeting group comprises a Fab that is engineered to introduce one or more buried interchain disulfide bonds. For example, in some embodiments, the Fab antibody comprises a heavy chain fragment that comprises F174C substitution, numbering according to Kabat, and/or a light chain fragment that comprises S176C substitution, numbering according to Kabat. In some embodiments, the Fab comprises a heavy chain fragment that comprises F174C and C233S substitutions, and a light chain fragment that comprises S176C and C214S substitutions, numbering according to Kabat. In some embodiments, the immune cell targeting group comprises a Fab that is engineered to knock out one or more natural interchain disulfide bonds, and to introduce one or more buried interchain disulfide bonds. In some embodiments, the immune cell targeting group comprises a Fab that comprises a cysteine at the C-terminus of the heavy or light chain fragment. In some embodiments, the Fab further comprises one or more amino acids between the heavy chain

fragment of the Fab and the C-terminal cysteine. In some embodiments, the Fab comprises a heavy chain variable domain linked to an antibody CH1 domain and a light chain variable domain linked to an antibody light chain constant domain, wherein the CH1 domain and the light chain constant domain are linked by one or more interchain disulfide bonds, and wherein the immune cell targeting group further comprises a single chain variable fragment (scFv) linked to the C-terminus of the light chain constant domain by an amino acid linker. In some embodiments, the Fab antibody is a DS Fab (Fab with wild type (natural) interchain disulfide bond), a NoDS Fab (Fab with natural disulfide bond knocked out, such as a Fab with C233S substitution on the heavy chain, and/or C214S substitution on the light chain, numbering according to Kabat), a bDS Fab (Fab without natural disulfide bond, and with introduced non-natural interchain buried disulfide bond, such as a Fab with F174C and C233S on the heavy chain, and/or S176C and C214S substitution on the light chain, numbering according to Kabat), or a bDS Fab-ScFv (a bDS Fab linked to a ScFv through a linker, such as (G4S)x (SEQ ID NO: 154)). In some embodiments, the immune cell targeting group comprises a Fab that has a non-natural interchain disulfide bond (e.g., an engineered, buried interchain disulfide bond).

In some embodiments, the ISV or Nanobody further comprises a spacer comprising one or more amino acids between the V_{HH} domain and the C-terminal cysteine. In some embodiments, the immune cell targeting group comprises two or more V_{HH} domains. In some embodiments, the two or more V_{HH} domains are linked by an amino acid linker. In some embodiments, the immune cell targeting group comprises a first V_{HH} domain linked to an antibody CH1 domain and a second V_{HH} domain linked to an antibody light chain constant domain, and wherein optionally the antibody CH1 domain and the antibody light chain constant domain are linked by one or more disulfide bonds. In some embodiments, the immune cell targeting group comprises a V_{HH} domain linked to an antibody CH1 domain, and/or the antibody CH1 domain is linked to an antibody light chain constant domain by one or more disulfide bonds. In some embodiments, the CH1 domain comprises F174C and C233S substitutions, and/or the light chain constant domain comprises S176C and C214S substitutions, numbering according to Kabat. In some embodiments, the antibody is a ScFv, a V_{HH} (Nb), a 2xV_{HH}, a V_{HH}-CH1/empty Vk, or a V_{HH}1-CH1/V_{HH}-2-Nb bDS.

In some embodiments, the immune cell targeting group comprises a Fab that comprises:

(a) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 1 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 2 or 3;

(b) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 4 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 5;

(c) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 6 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 7;

(d) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 8 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 9;

(e) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 10 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 11;

(f) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 12 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 13;

(g) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 14 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 15;

(h) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 16 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 17;

(i) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 18 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 19;

(j) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 20 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 21; or

(k) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 22 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 23.

In some embodiments, the immune cell targeting group comprise a bispecific targeting moiety. In some embodiments, the bispecific targeting moiety binds to the two different types of immune cells. In some embodiments, the two different types of immune cells are CD4+ T cells and CD8+

T cell. In some embodiments, the bispecific targeting moiety is a bispecific antibody. In some embodiments, the bispecific antibody is a Fab-ScFv. In some embodiments, the LNP binds to a first antigen on the surface of the first type of immune cell, and also binds to a second antigen on the surface of the second type of immune cell. In some embodiments, the LNP comprises two conjugates. In some embodiments, the first conjugate comprises a first antibody that binds to the first antigen of the first type of immune cell, and the second conjugate comprises a second antibody that binds to the second antigen of the second type of immune cell. In some embodiments, the two different types of immune cells are T cells and NK cells. In some embodiments, the immune cell targeting group binds to (i) both CD3 and CD56; (ii) both CD8 and CD56; or (iii) both CD7 and CD56.

As discussed herein, the LNPs may be targeted to a particular cell type, e.g., an immune cell, e.g., a T cell, B cell, or natural killer (NK) cell. This can be accomplished by using one or more of the lipids described herein. Furthermore, targeting can be enhanced by including a targeting group at a solvent accessible surface of an LNP particle. For example, targeting groups may include a member of a specific binding pair, e.g., an antibody-antigen pair, a ligand-receptor pair, etc. In certain embodiments, the targeting group is an antibody. Targeting can be implemented, for example, by using lipid-immune cell targeting group conjugates described herein.

Optionally, the targeting moiety is an antibody fragment without an Fc component. Previous attempts to target circulating immune cells with LNPs have employed full antibodies (WO 2016/189532 A1). Liposomes or lipid based particles with conjugated full antibodies clear more quickly from the circulation due to engagement of the Fc, reducing their potential for reaching the target cell of interest (Harding et al. (1997) Biochim Biophys. Acta 1327, 181-192; Sapra et al. (2004) Clin Cancer Res 10, 1100-1111; Aragnol et al., (1986) Proc Natl Acad Sci USA 83, 2699-2703). Liposomes targeted with antibody fragments retain their long circulating properties, like those targeted to EGFR (Mamot et al., (2005) Cancer Res 65, 11631-11638), ErbB2 (Park et al. (2002) Clin Cancer Res 8, 1172-1181), or EphA2 (Kamoun et al., 2019 Nat. Biomed. Eng 3, 264-280). In addition, lipid based carriers can be prepared using a micellar insertion process that allows for the nearly quantitative incorporation of the antibody conjugation following its separate

manufacturing (Nellis et al. (2005) Biotechnol Prog 21, 221-232), compared to a highly inefficient insertion when conjugating full IgGs (Ishida et al. (1999) FEBS Lett.460, 129-133) or the need to complete conjugation directly on an intact LNP (WO 2016/189532 Al). scFv, Fab, or VHH fragments can also be directly conjugated to activated PEG-lipids to make insertable conjugates.

In some embodiments, PEG-(lipid) is equivalent to (lipid)-PEG.

In certain embodiments, a targeting group may be a surface-bound antibody or surface bound antigen binding fragment thereof, which can permit tuning of cell targeting specificity. This is especially useful since highly specific antibodies can be raised against an epitope of interest for the desired targeting site. In one embodiment, multiple different antibodies can be incorporated into, and presented at the surface of an LNP, where each antibody binds to different epitopes on the same antigen or different epitopes on different antigens. Such approaches can increase the avidity and specificity of targeting interactions to a particular target cell.

A targeting group or combination of targeting groups can be selected based on the desired localization, function, or structural features of a given target cell. For example, in order to target a T-cell, T-cell population or T-cell subpopulation, one or more antibodies or antigen binding fragments or antigen binding derivatives thereof may be selected that target a T-cell, such as via a T-cell surface antigen. Exemplary T-cell surface antigens include, but are not limited to, for example, CD2, CD3, CD4, CD5, CD7, CD8, CD28, CD39, CD69, CD103, CD137, CD45, T-cell receptor (TCR) β, TCR-α, TCR-α/β, TCR-γ/δ, PD1, CTLA4, TIM3, LAG3, CD18, IL-2 receptor, CD11a, GL7, TLR2, TLR4, TLR5 and IL-15 receptor. In order to target an NK cell, or NK cell population, one or more antibodies, antigen binding fragments or antigen binding derivatives thereof may be selected that target an NK cell such as via a NK cell surface antigen. Exemplary NK cell surface antigens include, but are not limited to, CD48, CD56, CD85a, CD85c, CD85d, CD85e, CD85f, CD85i, CD85j, CD158b2, CD161, CD244, CD16a, CD16b, IL-2 receptor, CD27, CD28, CD48, CD69, CD70, CD86, CD112, CD122, CD155, CD161, CD244, CD266, CD314 / NKG2D, CD336 / NKP44, CD337 / NKP30. In order to target a B cell or B cell population, one or more antibodies, antigen binding fragments or antigen binding derivatives thereof may be selected that target a B cell such as via a B cell antigen. Exemplary B cell

antigens include, but are not limited to, CD19 for all B cells except plasma cells, CD19, CD25, and CD30 for activated B cells, CD27, CD38, CD78, CD138, and CD319 for plasma cells, CD20, CD27, CD40, CD80 and PDL-2 for memory cells, Notch2, CD1, CD21, and CD27 for marginal zone B cells, CD21, CD22, and CD23 for follicular B cells, and CD1, CD5, CD21, CD24, and TLR4 for regulatory B cells.

In certain embodiments, targeting can be implemented, for example, by using lipid-immune cell targeting group conjugates described herein. Exemplary lipid-immune cell targeting group conjugates can include compounds of Formula (II), [Lipid] – [optional linker] – [immune cell targeting group, e.g., T-cell targeting molecule, e.g., anti-CD2 antibody, anti-CD3 antibody, anti-CD7 antibody, or anti-CD8 antibody] (Formula II).

In some embodiments, the immune cell targeting group is a polypeptide, and the lipid is conjugated to the N-terminus, C-terminus, or anywhere in the middle part of the polypeptide.

In certain embodiments, the targeting group or targeting molecule is a T-cell targeting agent, for example, an antibody, that binds to a T-cell antigen selected from the group consisting of CD2, CD3, CD4, CD5, CD7, CD8, CD28, CD137, CD45, T-cell receptor (TCR)β,TCR-α, TCR-α/β,TCR-γ/δ, PD1, CTLA4, TIM3, LAG3, CD18, IL-2 receptor, CD11a, TLR2, TLR4, TLR5, IL-7 receptor, or IL-15 receptor. In certain embodiments, the T cell antigen may be CD2, and the targeting group can be, for example, an anti-CD2 antibody. In certain embodiments, the T cell antigen may be CD3, and the targeting group can be, for example, an anti-CD3 antibody. In certain embodiments, the T cell antigen may be CD4, and the targeting group can be, for example, an anti-CD4 antibody. In certain embodiments, the T cell antigen may be CD5, and the targeting group can be, for example, an anti-CD5 antibody. In certain embodiments, the T cell antigen may be CD7, and the targeting group can be, for example, an anti-CD7 antibody. In certain embodiments, the T cell antigen may be CD8, and the targeting group can be, for example, an anti-CD8 antibody. In certain embodiments, the T cell antigen may be TCR β, and the targeting group can be, for example, an anti-TCR β antibody. In some embodiments, the antibody is a human or humanized antibody.

In some embodiments, the LNP comprises two conjugates. In some embodiments, the first conjugate comprises an antibody that binds CD3. In some embodiments, the second conjugate

comprises an antibody that binds CD11a or CD18. In some embodiments, the LNP comprises one conjugate. In some embodiments, the conjugate comprises a bispecific antibody that binds both CD3 and CD11a. In some embodiments, the conjugate comprises a bispecific antibody that binds both CD3 and CD18. In some embodiments, the LNP binds CD7 and CD8 of the immune cell. In some embodiments, the first conjugate comprises an antibody that binds CD7, and a second conjugate that binds CD8. In some embodiments, the LNP comprises one conjugate. In some embodiments, the conjugate comprises a bispecific antibody that binds CD7 and CD8.

An exemplary CD2 binding agent can be an antibody selected from the group consisting of 9.6 (academic.oup.com/intimm/article/10/12/1863/744536), 9-1 (academic.oup.com/intimm/article/10/12/1863/744536), TS2/18.1.1 (ATCC HB-195), Lo-CD2b (ATCC PTA-802), Lo-CD2a/BTI-322 (US Patent 6849258B1), Sipilzumab/MEDI-507 (US Patent 6849258B1/en), 35.1 (ATCC HB-222), OKT11 (ATCC CRL-8027), RPA-2.1 (PCT Publication WO2020023559A1), AF1856 (R&D Systems), MAB18562 (R&D Systems), MAB18561 (R&D Systems), MAB1856 (R&D Systems), PAB30359 (Abnova Corporation), 10299-1 (Abnova Corporation), and antigen binding fragments thereof. In certain embodiments, the binding agent comprises a heavy chain variable domain (V_H) and a light chain variable domain (V_L) of an antibody selected from the group consisting of AF1856 (R&D Systems), MAB18562 (R&D Systems), MAB18561 (R&D Systems), MAB1856 (R&D Systems), PAB30359 (Abnova Corporation), and 10299-1 (Abnova Corporation). In certain embodiments, the binding agent comprises the heavy chain CDR_1, CDR_2, and CDR_3 and the light chain CDR_1, CDR_2, and CDR_3, determined under Kabat (see, Kabat et al., (1991) Sequences of Proteins of Immunological Interest, NIH Publication No. 91-3242, Bethesda), Chothia (see, e.g., Chothia C & Lesk A M, (1987), J. MOL. BIOL.196: 901-917), MacCallum (see, MacCallum R M et al., (1996) J. MOL. BIOL. 262: 732-745), or any other CDR determination method known in the art, of the V_H and V_L sequences of an antibody selected from the group consisting of AF1856 (R&D Systems), MAB18562 (R&D Systems), MAB18561 (R&D Systems), MAB1856 (R&D Systems), PAB30359 (Abnova Corporation), and 10299-1 (Abnova Corporation).

An exemplary CD2 binding agent can also be selected from antibodies or antibody fragments employing CDRs of clones 9.6, 9-1, TS2/18.1.1, Lo-CD2b, Lo-CD2a, BTI-322, sipilzumab,

35.1, OKT11, RPA-2.1, SQB-3.21, LT2, TS1/8, UT329, 4F22, OX-34, UQ2/42, MU3, U7.4, NFN-76, or MOM-181-4-F(E).

An exemplary CD3 binding agent (CD3γ/δ/ε, CD3γ, CD3δ, CD3γ/ε, CD3δ/ε, or CD3ε) can be an antibody selected from the group consisting of MEM-57 (CD3γ/δ/ε, EnzoLife Sciences), MAB100 (CD3ε, R&D Systems), CD3-H5 (CD3ε, Abnova Corporation), CD3-12 (CD3ε, Cell Signaling Technology), LE-CD3 (CD3ε, Santa Cruz Biotechnology, Inc.), NBP1-31250 (CD3γ, Novus Biologicals), 16669-1-AP (CD3δ, Invitrogen) and antigen binding fragments thereof. In certain embodiments, the binding agent comprises a V_H domain and a V_L domain of an antibody selected from the group consisting of MEM-57 (CD3γ/δ/ε, EnzoLife Sciences), MAB100 (CD3ε, R&D Systems), CD3-H5 (CD3ε, Abnova Corporation), CD3-12 (CD3ε, Cell Signaling Technology), LE-CD3 (CD3ε, Santa Cruz Biotechnology, Inc.), NBP1-31250 (CD3γ, Novus Biologicals), and 16669-1-AP (CD3δ, Invitrogen). In certain embodiments, the binding agent comprises the heavy chain CDR_1, CDR_2, and CDR_3 and the light chain CDR_1, CDR_2, and CDR_3, determined under Kabat (see, Kabat et al., (1991) Sequences of Proteins of Immunological Interest, NIH Publication No. 91-3242, Bethesda), Chothia (see, e.g., Chothia C & Lesk A M, (1987), J. MOL. BIOL.196: 901-917), MacCallum (see, MacCallum R M et al., (1996) J. MOL. BIOL. 262: 732-745), or any other CDR determination method known in the art, of the V_H and V_L sequences of an antibody selected from the group consisting of MEM-57 (CD3γ/δ/ε, EnzoLife Sciences), MAB100 (CD3ε, R&D Systems), CD3-H5 (CD3ε, Abnova Corporation), CD3-12 (CD3ε, Cell Signaling Technology), LE-CD3 (CD3ε, Santa Cruz Biotechnology, Inc.), NBP1-31250 (CD3γ, Novus Biologicals), and 16669-1-AP (CD3δ, Invitrogen).

An exemplary CD3 binding agent can also be selected from antibodies or antibody fragments employing CDRs of clones hsp34, OKT-3, UCHT1, 38.1, HIT3a, RFT8, SK7, BC3, SP34-2, HU291, TRX4, Catumaxomab, teplizumab, 3-106, 3-114, 3-148, 3-190, 3-271, 3-550, 4-10, 4-48, H2C, F12Q, I2C, SP7, 3F3A1, CD3-12, 301, RIV9, JB38-29, JE17-74, GT0013, 4E2, 7A4, 4D10A6, SPV-T3b, M2AB, ICO-90, 30A1 or Hu38E4.v1 (US Patent Application 20200299409A1), REGN5458 (US Patent Application 20200024356A1), Blinatumomab (go.drugbank.com/drugs/DB09052/polypeptide_sequences.fasta). In some embodiments, the conjugate comprises a Fab, wherein the Fab comprises (a) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 1 and a light chain fragment comprising the amino acid

sequence of SEQ ID NO: 2 or 3. In some embodiments, the LNP binds CD3, and also binds CD11a or CD18.

An exemplary CD4 binding agent can be an antibody selected from the group consisting of Ibalizumab (www.genome.jp/dbget-bin/www_bget?D09575), AF1856 (R&D Systems), MAB554 (R&D Systems), BF0174 (Affinity Biosciences), PAB31115 (Abnova Corporation), CAL4 (Abcam), and antigen binding fragments thereof. In certain embodiments, the binding agent comprises a V_H domain and a V_L domain of an antibody selected from the group consisting of AF1856 (R&D Systems), MAB554 (R&D Systems), BF0174 (Affinity Biosciences), PAB31115 (Abnova Corporation), and CAL4 (Abcam). In certain embodiments, the binding agent comprises the heavy chain CDR_1, CDR_2, and CDR_3 and the light chain CDR_1, CDR_2, and CDR_3, determined under Kabat (see, Kabat et al., (1991) Sequences of Proteins of Immunological Interest, NIH Publication No. 91-3242, Bethesda), Chothia (see, e.g., Chothia C & Lesk A M, (1987), J. MOL. BIOL.196: 901-917), MacCallum (see, MacCallum R M et al., (1996) J. MOL. BIOL. 262: 732-745), or any other CDR determination method known in the art, of the V_H and V_L sequences of an antibody selected from the group consisting of AF1856 (R&D Systems), MAB554 (R&D Systems), BF0174 (Affinity Biosciences), PAB31115 (Abnova Corporation), and CAL4 (Abcam).

An exemplary CD4 binding agent can also be selected from antibodies or antibody fragments employing CDRs of clones Ibalizumab, OKT4, RPA-T4, S3.5, SK3, N1UG0, RIV6, OTI18E3, MEM-241, B486A1, RFT-4g, 7E14, MDX.2, MEM-115, MEM-16, ICO-86, Edu-2, or ilbalizumab.

An exemplary CD5 binding agent can be an antibody selected from the group consisting of He3, MAB1636 (R&D Systems), AF1636 (R&D Systems), MAB115 (R&D Systems), C5/473 + CD5/54/F6 (Abcam), CD5/54/F6 (Abcam), 65152 (Proteintech), and antigen binding fragments thereof. In some embodiments, the binding agent comprises a V_H domain and a V_L of an antibody selected from the group consisting of MAB1636 (R&D Systems), AF1636 (R&D Systems), MAB115 (R&D Systems), C5/473 + CD5/54/F6 (Abcam), CD5/54/F6 (Abcam), and 65152 (Proteintech). In certain embodiments, the binding agent comprises the heavy chain CDR_1, CDR_2, and CDR_3 and the light chain CDR_1, CDR_2, and CDR_3, determined under Kabat

(see, Kabat et al., (1991) Sequences of Proteins of Immunological Interest, NIH Publication No.91-3242, Bethesda), Chothia (see, e.g., Chothia C & Lesk A M, (1987), J. MOL. BIOL. 196: 901-917), MacCallum (see, MacCallum R M et al., (1996) J. MOL. BIOL. 262: 732-745), or any other CDR determination method known in the art, of the V_H and V_L sequences of an antibody selected from the group consisting of MAB1636 (R&D Systems), AF1636 (R&D Systems), MAB115 (R&D Systems), C5/473 + CD5/54/F6 (Abcam), CD5/54/F6 (Abcam), and 65152 (Proteintech).

An exemplary CD5 binding agent can also be selected from antibodies or antibody fragments employing CDRs of clones of zolimomab, 5D7, L17F12, and UCHT2, 1D8, 3I21, 4H10, 8J23, 5O4, 4H2, 5G2, 8G8, 6M4, 2E3, 4E24, 4F10, 7J9, 7P9, 8E24, 6L18, 7H7, 1E7, 8J21, 7I11, 8M9, 1P21, 2H11, 3M22, 5M6, 5H8, 7I19, 1A2, 8E15, 8C10, 3P16, 4F3, 5M24, 5O24, 7B16, 1E8, 2H16, BLa1, 1804, DK23, Cris1, MEM-32, H65, 4C7, OX-19, Leu-1, 53-7.3, 4H8E6, T101, EP2952, D-9, H-3, HK231, N-20, Y2/178, H-300, CD5/54/F6, Q-20, CC17, MOM-18539-S(P), or MOM-18885-S(P).

An exemplary CD7 binding agent can be an antibody selected from the group consisting of MAB7579 (R&D Systems), AF7579 (R&D Systems), EPR22065 (Abcam), 1G10D8 (Proteintech), NBP2-32097 (Novus Biologicals), NBP2-38440 (Novus Biologicals), and antigen binding fragments thereof. In certain embodiments, the binding agent comprises a V_H domain and a V_L of an antibody selected from the group consisting of MAB7579 (R&D Systems), AF7579 (R&D Systems), EPR22065 (Abcam), 1G10D8 (Proteintech), NBP2-32097 (Novus Biologicals), and NBP2-38440 (Novus Biologicals). In certain embodiments, the binding agent comprises the heavy chain CDR_1, CDR_2, and CDR_3 and the light chain CDR_1, CDR_2, and CDR_3, determined under Kabat (see, Kabat et al., (1991) Sequences of Proteins of Immunological Interest, NIH Publication No.91-3242, Bethesda), Chothia (see, e.g., Chothia C & Lesk A M, (1987), J. MOL. BIOL. 196: 901-917), MacCallum (see, MacCallum R M et al., (1996) J. MOL. BIOL. 262: 732-745), or any other CDR determination method known in the art, of the V_H and V_L sequences of an antibody selected from the group consisting of MAB7579 (R&D Systems), AF7579 (R&D Systems), EPR22065 (Abcam), 1G10D8 (Proteintech), NBP2-32097 (Novus Biologicals), and NBP2-38440 (Novus Biologicals).

An exemplary CD7 binding agent can also be selected from antibodies or antibody fragments employing CDRs of clones TH-69, 3Af11, T3-3A1, 124-1D1, 3A1f, CD7-6B7, or VHH6.

An exemplary CD8 (CD8α, CD8α/α, CD8α/β or CD8β) binding agent can be an antibody selected from the group consisting of 2.43 (Invitrogen), Du CD8-1 (CD8α, Invitrogen), 9358-CD (CD8α/β, R&D Systems), MAB116 (CD8α, R&D Systems), ab4055 (CD8α, Abcam), C8/144B (CD8α, Novus Biologicals), YTS105.18 (CD8α, Novus Biologicals), TRX2 patents.justia.com/patent/20170198045), and antigen binding fragments thereof. In certain embodiments, the binding agent comprises a V_H domain and a V_L domain of an antibody selected from the group consisting of 2.43 (Invitrogen), 51.1 (ATCC HB-230), Du CD8-1 (CD8α, Invitrogen), 9358-CD (CD8α/β, R&D Systems), MAB116 (CD8α, R&D Systems), ab4055 (CD8α, Abcam), C8/144B (CD8α, Novus Biologicals), and YTS105.18 (CD8α, Novus Biologicals). In certain embodiments, the binding agent comprises the heavy chain CDR_1, CDR_2, and CDR_3 and the light chain CDR_1, CDR_2, and CDR_3, determined under Kabat (see, Kabat et al., (1991) Sequences of Proteins of Immunological Interest, NIH Publication No.91-3242, Bethesda), Chothia (see, e.g., Chothia C & Lesk A M, (1987), J. MOL. BIOL.196: 901-917), MacCallum (see, MacCallum R M et al., (1996) J. MOL. BIOL.262: 732-745), or any other CDR determination method known in the art, of the V_H and V_L sequences of an antibody selected from the group consisting of 2.43 (Invitrogen), Du CD8-1 (CD8α, Invitrogen), 9358-CD (CD8α/β, R&D Systems), MAB116 (CD8α, R&D Systems), ab4055 (CD8α, Abcam), C8/144B (CD8α, Novus Biologicals), and YTS105.18 (CD8α, Novus Biologicals).

An exemplary CD8 binding agent can also be selected from antibodies or antibody fragments employing CDRs of clones OKT-8, 51.1, S6F1, TRX2, and UCHT4, SP16, 3B5, C8-144B, HIT8a, RAVB3, LT8, 17D8, MEM-31, MEM-87, RIV11, DK-25, YTC141.1HL, or YTC182.20. In some embodiments, the conjugate comprises a Fab, wherein the Fab comprises a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 6 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 7.

An exemplary CD8 binding agent is an antibody (e.g., an ISV) that comprises three complementarity determining domains CDR1, CDR2, and CDR3. In some embodiments, the CDR1 comprises GSTFSDYG (SEQ ID NO: 100) or an amino acid sequence that has at least

80%, at least 90%, at least 95%, at least 99% or more sequence identity thereto. In some embodiments, the CDR2 comprises IDWNGEHT (SEQ ID NO: 101) or an amino acid sequence that has at least 80%, at least 90%, at least 95%, at least 99% or more sequence identity thereto. In some embodiments, the CDR3 comprises AADALPYTVRKYNY (SEQ ID NO: 102) or an amino acid sequence that has at least 80%, at least 90%, at least 95%, at least 99% or more sequence identity thereto. In some embodiments: (1) any amino acid substitution is a conservative amino acid substitution; and/or (2) said amino acid sequence only contains amino acids substitutions, and no amino acid deletions or insertions relative thereto. In some embodiments, the ISVD comprises, consists of, or consists essentially of SEQ ID NO: 77. In some embodiments, the agent has at least 80%, at least 85%, such as 90% or 95% or more sequence identity with SEQ ID NO: 77, or 3, 2, or 1 amino acid difference with SEQ ID NO: 77; or any suitable combination thereof. In some embodiments, the CD8 is derived from a mammalian animal, such as a human being.

In one embodiment, the CD8 Nanobody is BDSn:

Anti-CD8 BDSn Nb sequence (CDR1, CDR2, CDR3 underlined based on IMGT designation):

EVQLVESGGGLVQAGGSLRLSCAASGSTFSDYGVGWFRQAPGKGREFVADIDWNGEHT SYADSVKGRFATSRDNAKNTAYLQMNSLKPEDTAVYYCAADALPYTVRKYNY WGQGTQVTVSSGGCGGHHHHHH (SEQ ID NO: 77)

In some embodiments, a CD8 Nanobody described herein binds to CD8 with an dissociation constant (KD) of 10^{-5} to 10^{-12} moles/liter (M) or less, 10^{-7} to 10−12 moles/liter (M) or less, 10^{-8} to 10^{-12} moles/liter (M), and/or with an association constant (KA) of at least 10^{7} M^{-1}, at least 10^{8} M^{-1}, at least 10^{9} M^{-1}, such as at least 10^{12} M^{-1}; and in particular with a KD less than 500 nM, less than 200 nM, less than 10 nM, such as less than 500 μM. The KD and KA values of a nanobody disclosed herein against vWF can be determined. More generally, the nanobodies described herein may have a dissociation constant with respect to vWF that is as described in this paragraph.

One class of CD8 Nanobodies of the disclosure comprises Nanobodies with an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring VHH domain, but

that has been "humanized", i.e. by replacing one or more amino acid residues in the amino acid sequence of said naturally occurring VHH sequence by one or more of the amino acid residues that occur at the corresponding position(s) in a VH domain from a conventional 4-chain antibody from a human being (e.g., indicated above). It should be noted that such humanized CD8 Nanobodies of the present disclosure can be obtained in any suitable manner known per se (i.e. as indicated under points (1)-(8) above) and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring VHH domain as a starting material.

Another class of CD8 Nanobodies of the present disclosure comprises Nanobodies with an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring VH domain that has been "camelized", i.e. by replacing one or more amino acid residues in the amino acid sequence of a naturally occurring VH domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a VHH domain of a heavy chain antibody. This can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the further description below. Reference is also made to WO 94/04678. Such camelization may preferentially occur at amino acid positions which are present at the VH-VL interface and at the so-called Camelidae hallmark residues (see for example also WO 94/04678), as also mentioned below. In some embodiments, the VH domain or sequence that is used as a starting material or starting point for generating or designing the camelized Nanobody is a VH sequence from a mammal, e.g.,VH sequence of a human being. It should be noted that such camelized Nanobodies of the present disclosure can be obtained in any suitable manner known per se and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring VH domain as a starting material.

For example, both "humanization" and "camelization" can be performed by providing a nucleotide sequence that encodes such a naturally occurring VHH domain or VH domain, respectively, and then changing, in a manner known per se, one or more codons in said nucleotide sequence such that the new nucleotide sequence encodes a humanized or camelized Nanobody of the present disclosure, respectively, and then expressing the nucleotide sequence thus obtained in a manner known per se so as to provide the desired Nanobody. Alternatively,

based on the amino acid sequence of a naturally occurring VHH domain or VH domain, respectively, the amino acid sequence of the desired humanized or camelized Nanobody of the present disclosure, respectively, can be designed and then synthesized de novo using techniques for peptide synthesis known per se. Also, based on the amino acid sequence or nucleotide sequence of a naturally occurring VHH domain or VH domain, respectively, a nucleotide sequence encoding the desired humanized or camelized Nanobody can be designed and then synthesized de novo using techniques for nucleic acid synthesis known per se, after which the nucleotide sequence thus obtained can be expressed in a manner known per se so as to provide the desired Nanobody.

Other suitable ways and techniques for obtaining Nanobodies and/or nucleotide sequences and/or nucleic acids encoding the same, starting from (the amino acid sequence of) naturally occurring VH domains or preferably VHH domains and/or from nucleotide sequences and/or nucleic acid sequences encoding the same will be clear from the skilled person, and may for example comprising combining one or more amino acid sequences and/or nucleotide sequences from naturally occurring VH domains (such as one or more FR's and/or CDR's) with one or more one or more amino acid sequences and/or nucleotide sequences from naturally occurring VHH domains (such an one or more FR's or CDR's), in a suitable manner so as to provide (a nucleotide sequence or nucleic acid encoding) a Nanobody. Also provided are compounds and constructs, and in particular proteins and polypeptides that comprise or essentially consists of at least one such amino acid sequence and/or Nanobody of the disclosure (or suitable fragments thereof), and optionally further comprises one or more other groups, residues, moieties or binding units. In some embodiments, such further groups, residues, moieties, binding units or amino acid sequences may or may not provide further functionality to the amino acid sequence and/or Nanobody (and/or to the compound or construct in which it is present) and may or may not modify the properties of the amino acid sequence and/or Nanobody.

The disclosure also encompasses any polypeptide of the present disclosure that has been glycosylated at one or more amino acid positions, usually depending on the host used to express the polypeptide. A polypeptide can comprise an amino acid sequence of a CD8 Nanobody of the present disclosure, which is fused at its amino terminal end, at its carboxy terminal end, or both at its amino terminal end and at its carboxy terminal end with at least one further amino acid

sequence. Such further amino acid sequence may comprise at least one further Nanobody, so as to provide a polypeptide that comprises at least two, such as three, four or five, Nanobodies, in which said Nanobodies may optionally be linked via one or more linker sequences (as defined herein). Polypeptides of comprising CD8 Nanobody of the present disclosure and one or more another Nanobodies are multivalent polypeptides. In a multivalent polypeptide, the two or more Nanobodies may be the same or different. For example, the two or more Nanobodies in a multivalent polypeptide:

• may be directed against the same antigen, i.e. against the same parts or epitopes of said antigen or against two or more different parts or epitopes of said antigen; and/or:

• may be directed against the different antigens;

• or a combination thereof.

Thus, a bivalent polypeptide, for example:

• may comprise two identical Nanobodies;

• may comprise a first Nanobody directed against a first part or epitope of an antigen and a second Nanobody directed against the same part or epitope of said antigen or against another part or epitope of said antigen;

or may comprise a first Nanobody directed against a first antigen and a second Nanobody directed against a second antigen different from said first antigen;

whereas a trivalent Polypeptide of the Invention for example:

• may comprise three identical or different Nanobodies directed against the same or different parts or epitopes of the same antigen;

• may comprise two identical or different Nanobodies directed against the same or different parts or epitopes on a first antigen and a third Nanobody directed against a second antigen different from said first antigen; or

• may comprise a first Nanobody directed against a first antigen, a second Nanobody directed against a second antigen different from said first antigen, and a third Nanobody directed against a third antigen different from said first and second antigen.

An exemplary CD137 binding agent can be selected from antibodies or antibody fragments employing CDRs of clones 4B4-1, P566, or Urelumab. An exemplary CD28 binding agent can be selected from antibodies or antibody fragments employing CDRs of clone TAB08. An exemplary CD45 binding agent can be selected from antibodies or antibody fragments employing CDRs of clones BC8, 9.4, 4B2, Tu116, or GAP8.3. An exemplary CD18 binding agent can be selected from antibodies or antibody fragments employing CDRs of clones 1B4, TS1/18, MEM-48, YFC118-3, TA-4, MEM-148, or R3-3, 24. An exemplary CD11a binding agent can be selected from antibodies or antibody fragments employing CDRs of clone MHM24 or Efalizumab. An exemplary IL-2 receptor binding agent can be selected from of antibodies or antibody fragments employing CDRs of clones YTH 906.9HL, IL2R.1, BC96, B-B10, 216, MEM-181, ITYV, MEM-140, ICO-105, Daclizumab, or from the group consisting of IL2 or fragments of IL2. An exemplary IL-15R binding agent can be selected from antibodies or antibody fragments employing CDRs of clones JM7A4, or OTI3D5, or from the group consisting of IL15 or fragments of IL15. An exemplary TLR2 binding agent can be selected from antibodies or antibody fragments employing CDRs of clones JM22-41, TL2.1, 11G7, or TLR2.45. An exemplary TLR4 binding agent can be selected from antibodies or antibody fragments employing CDRs of clones HTA125, or 76B357-1. An exemplary TLR5 binding agent can be selected from antibodies or antibody fragments employing CDRs of clones 85B152-5, or 9D759-2. An exemplary GL7 binding agent can be selected from antibodies or antibody fragments employing CDRs of clone GL7.

An exemplary PD1 binding agent can be selected from antibodies or antibody fragments employing CDRs of clones MIH4, J116, J150, OTIB11, OTI17B10, OTI3A1, or OTI16D4. In addition, exemplary anti-PD-1 antibodies are described, for example, in U.S. Patent Nos. 8,952,136, 8,779,105, 8,008,449, 8,741,295, 9,205,148, 9,181,342, 9,102,728, 9,102,727, 8,952,136, 8,927,697, 8,900,587, 8,735,553, and 7,488,802. Exemplary anti-PD-1 antibodies

include, for example, nivolumab (Opdivo®, Bristol-Myers Squibb Co.), pembrolizumab (Keytruda®, Merck Sharp & Dohme Corp.), PDR001 (Novartis Pharmaceuticals), and pidilizumab (CT-011, Cure Tech). Exemplary anti-PD-L1 antibodies are described, for example, in U.S. Patent Nos. 9,273,135, 7,943,743, 9,175,082, 8,741,295, 8,552,154, and 8,217,149. Exemplary anti-PD-L1 antibodies include, for example, atezolizumab (Tecentriq®, Genentech), durvalumab (AstraZeneca), MEDI4736, avelumab, and BMS 936559 (Bristol Myers Squibb Co.).

An exemplary CTLA-4 binding agent can be selected from antibodies or antibody fragments employing CDRs of clones ER4.7G.11 [7G11], OTI9G4, OTI9F3, OTI3A5, A3.4H2.H12, 14D3, OTI3A12, OTI1A11, OTI1E8, OTI3B11, OTI3D2, OTI10C8, OTI2E9, OTI6F1, OTI7D3, OTI85B, OTI12C6. Exemplary anti-CTLA-4 antibodies are described in U.S. Patent Nos.6,984,720, 6,682,736, 7,311,910; 7,307,064, 7,109,003, 7,132,281, 6,207,156, 7,807,797, 7,824,679, 8,143,379, 8,263,073, 8,318,916, 8,017,114, 8,784,815, and 8,883,984, International (PCT) Publication Nos. WO98/42752, WO00/37504, and WO01/14424, and European Patent No. EP 1212422 B1. Exemplary CTLA-4 antibodies include ipilimumab or tremelimumab.

An exemplary TCR β binding agent can be an antibody selected from the group consisting of H57-597 (Invitrogen), 8A3 (Novus Biologicals), R73 (TCRα/ β, Abcam), E6Z3S (TRBC1/TCRβ, Cell Signaling Technology), and antigen binding fragments thereof. In certain embodiments, the binding agent comprises a V_H domain and a V_L of an antibody selected from the group consisting of H57-597 (Invitrogen), 8A3 (Novus Biologicals), R73 (TCRα/ β, Abcam), and E6Z3S (TRBC1/TCRβ, Cell Signaling Technology). In certain embodiments, the binding agent comprises the heavy chain CDR_1, CDR_2, and CDR_3 and the light chain CDR_1, CDR_2, and CDR_3, determined under Kabat (see, Kabat et al., (1991) Sequences of Proteins of Immunological Interest, NIH Publication No.91-3242, Bethesda), Chothia (see, e.g., Chothia C & Lesk A M, (1987), J. MOL. BIOL. 196: 901-917), MacCallum (see, MacCallum R M et al., (1996) J. MOL. BIOL. 262: 732-745), or any other CDR determination method known in the art, of the V_H and V_L sequences of an antibody selected from the group consisting of H57-597 (Invitrogen), 8A3 (Novus Biologicals), R73 (TCRα/ β, Abcam), and E6Z3S (TRBC1/TCRβ, Cell Signaling Technology).

An exemplary CD137 binding agent can be selected from antibodies or antibody fragments employing CDRs of clones 4B4-1, P566, or Urelumab.

In some embodiments, the immune cell targeting group comprises an antibody selected from the group consisting of a Fab, F(ab')2, Fab'-SH, Fv, and scFv fragment. In some embodiments, the antibody is a human or humanized antibody. In some embodiments, the immune cell targeting group comprises a Fab or an immunoglobulin single variable domain, such as a Nanobody. In some embodiments, the immune cell targeting group comprises a Fab that does not comprise a natural interchain disulfide bond. For example, in some embodiments, the Fab comprises a heavy chain fragment that comprises a C233S substitution, and/or a light chain fragment that comprises a C214S substitution, numbering according to Kabat. In some embodiments, the immune cell targeting group comprises a Fab that comprises one or more non-native interchain disulfide bonds. In some embodiments, the interchain disulfide bonds are between two non-native cysteine residues on the light chain fragment and heavy chain fragment, respectively. For example, in some embodiments, the Fab comprises a heavy chain fragment that comprises F174C substitution, and/or a light chain fragment that comprises S176C substitution, numbering according to Kabat. In some embodiments, the Fab comprises a heavy chain fragment that comprises F174C and C233S substitutions, and/or a light chain fragment that comprises S176C and C214S substitutions, numbering according to Kabat. In some embodiments, the immune cell targeting group comprises a Fab lacking the native interchain disulfide bond. In some embodiments, the Fab is engineered to replace one or both cysteines on the native constant light chain and the native constant heavy chain that form the native interchain disulfide with a non-cysteine amino acid, therefor to remove the native interchain disulfide bond in the Fab. In some embodiments, the immune cell targeting group comprises a C-terminal cysteine residue. In some embodiments, the immune cell targeting group comprises a Fab that comprises a cysteine at the C-terminus of the heavy or light chain fragment. In some embodiments, the Fab further comprises one or more amino acids between the heavy chain of the Fab and the C-terminal cysteine. For example, in some embodiments, the Fab comprises two or more amino acids derived from an antibody hinge region (e.g., a partial hinge sequence) between the C-terminus of the Fab and the C-terminal cysteine. In some embodiments, the Fab comprises a heavy chain variable domain linked to an antibody CH1 domain and a light chain variable domain linked to an antibody light chain constant domain, wherein the CH1 domain and the light chain constant

domain are linked by one or more interchain disulfide bonds, and wherein the immune cell targeting group further comprises a single chain variable fragment (scFv) linked to the C-terminus of the light chain constant domain by an amino acid linker. In some embodiments, the Fab antibody is a DS Fab, a NoDS Fab, a bDS Fab, a bDS Fab-ScFv.

In some embodiments, the immune cell targeting group comprises an immunoglobulin single variable domain, such as a Nanobody (e.g., a V_{HH}). In some embodiments, the Nanobody comprises a cysteine at the C-terminus. In some embodiments, the Nanobody further comprises a spacer comprising one or more amino acids between the V_{HH} domain and the C-terminal cysteine. In some embodiments, the spacer comprises one or more glycine residues, e.g., two glycine residues. In some embodiments, the immune cell targeting group comprises two or more V_{HH} domains. In some embodiments, the two or more V_{HH} domains are linked by an amino acid linker. In some embodiments, the amino acid linker comprises one or more glycine and/or serine residues (e.g., one or more repeats of the sequence GGGGS (SEQ ID NO: 154)). In some embodiments, the immune cell targeting group comprises a first V_{HH} domain linked to an antibody CH1 domain and a second V_{HH} domain linked to an antibody light chain constant domain, and wherein the antibody CH1 domain and the antibody light chain constant domain are linked by one or more disulfide bonds (e.g., interchain disulfide bonds). In some embodiments, the immune cell targeting group comprises a V_{HH} domain linked to an antibody CH1 domain, and wherein the antibody CH1 domain is linked to an antibody light chain constant domain by one or more disulfide bonds. In some embodiments, the CH1 domain comprises F174C and C233S substitutions, and the light chain constant domain comprises S176C and C214S substitutions, numbering according to Kabat. In some embodiments, the antibody is a ScFv, a V_{HH}, a 2xV_{HH}, a V_{HH}-CH1/empty Vk, or a V_{HH}1-CH1/V_{HH}-2-Nb bDS.

An exemplary targeting moiety may have an amino sequence as set forth below:

Anti-CD3 hSP34-Fab sequences:

hSP34 heavy chain (HC) sequence (SEQ ID NO: 1):

EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKY
NNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISY

WAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSSDKTHTC

hSP34-mlam light chain (LC) sequence (mouse lambda) (SEQ ID NO: 2):

QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLA
PGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGQPKSS
PSVTLFPPSSEELETNKATLVCTITDFYPGVVTVDWKVDGTPVTQGMETTQPSKQS
NNKYMASSYLTLTARAWERHSSYSCQVTHEGHTVEKSLSRADSS

SP34-hlam LC (human lambda) (SEQ ID NO: 3):

QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLA
PGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLSQPK
AAPSVTLFPPSSEELQANKATLVCLVSDFYPGAVTVAWKADGSPVKVGVETTKPSK
QSNNKYAASSYLSLTPEQWKSHRSYSCRVTHEGSTVEKTVAPAESS

Anti-CD3 Hu291-Fab sequences:

Hu291 HC (SEQ ID NO: 4):

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGYINPRS
GYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCARSAYYDYDGFAYW
GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDK
THTC

Hu 291 LC (SEQ ID NO: 5):

MDMRVPAQLLGLLLLWLPGAKCDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNW
YQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSS
NPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGES

Anti-CD8 TRX2-Fab sequences:

TRX2 HC (SEQ ID NO: 6):

QVQLVESGGGVVQPGRSLRLSCAASGFTFSDFGMNWVRQAPGKGLEWVALIYYDG
SNKFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPHYDGYYHFFDS
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSD
KTHTC

TRX2 LC (SEQ ID NO: 7):

DIQMTQSPSSLSASVGDRVTITCKGSQDINNYLAWYQQKPGKAPKLLIYNTDILHTG
VPSRFSGSGSGTDFTFTISSLQPEDIATYYCYQYNNGYTFGQGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD8 OKT8-Fab sequences:

OKT8 HC (SEQ ID NO: 8):

QVQLVQSGAEDKKPGASVKVSCKASGFNIKDTYIHWVRQAPGQGLEWMGRIDPAN
DNTLYASKFQGRVTITADTSSNTAYMELSSLRSEDTAVYYCGRGYGYYVFDHWGQ
GTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTC

OKT8 LC (SEQ ID NO: 9):

DIVMTQSPSSLSASVGDRVTITCRTSRSISQYLAWYQEKPGKAPKLLIYSGSTLQSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQHNENPLTFGQGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD4 Ibalizumab-Fab sequences:

Ibalizumab HC (SEQ ID NO: 10):

QVQLQQSGPEVVKPGASVKMSCKASGYTFTSYVIHWVRQKPGQGLDWIGYINPYND
GTDYDEKFKGKATLTSDTSTSTAYMELSSLRSEDTAVYYCAREKDNYATGAWFAY
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSD
KTHTC

Ibalizumab LC (SEQ ID NO: 11):

DIVMTQSPDSLAVSLGERVTMNCKSSQSLLYSTNQKNYLAWYQQKPGQSPKLLIYW
ASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQYYSYRTFGGGTKLEIKRT
VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

anti-CD5 He3-Fab sequences:

He3 HC (SEQ ID NO: 12):

EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHT
GEPTYADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGYDWYFDVWGQG
TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTC

He3 LC (SEQ ID NO: 13):

DIQMTQSPSSLSASVGDRVTITCRASQDINSYLSWFQQKPGKAPKTLIYRANRLESGVP
SRFSGSGSGTDYTLTISSLQYEDFGIYYCQQYDESPWTFGGGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

anti-CD7 TH-69-Fab sequences:

TH-69 HC (SEQ ID NO: 14):

EVQLVESGGGLVKPGGSLKLSCAASGLTFSSYAMSWVRQTPEKRLEWVASISSGGFT
YYPDSVKGRFTISRDNARNILYLQMSSLRSEDTAMYYCARDEVRGYLDVWGAGTTV

TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC

TH-69 LC (SEQ ID NO: 15):

DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWYQQKPDGTVKLLIYYTSSLHSGVP
SRFSGSGSGTDYSLTISNLEPEDIATYYCQQYSKLPYTFGGGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

anti-CD2 TS2/18.1-Fab sequences:

TS2/18.1 HC (SEQ ID NO: 16):

EVQLVESGGGLVMPGGSLKLSCAASGFAFSSYDMSWVRQTPEKRLEWVAYISGGGF
TYYPDTVKGRFTLSRDNAKNTLYLQMSSLKSEDTAMYYCARQGANWELVYWGQGT
LVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTC

TS2/18.1 LC (SEQ ID NO: 17):

DIVMTQSPATLSVTPGDRVFLSCRASQSISDFLHWYQQKSHESPRLLIKYASQSISGIPS
RFSGSGSGSDFTLSINSVEPEDVGVYFCQNGHNFPPTFGGGTKLEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS
TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

anti-CD29.6-Fab sequences:

9.6 HC (SEQ ID NO: 18):

QVQLQQPGAELVRPGSSVKLSCKASGYTFTRYWIHWVKQRPIQGLEWIGNIDPSDSE
THYNQKFKDKATLTVDKSSGTAYMQLSSLTSEDSAVYYCATEDLYYAMEYWGQGT
SVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTC

9.6 LC (SEQ ID NO: 19):

NIMMTQSPSSLAVSAGEKVTMTCKSSQSVLYSSNQKNYLAWYQQKPGQSPKLLIYW
ASTRESGVPDRFTGSGSGTDFTLTISSVQPEDLAVYYCHQYLSSHTFGGGTKLEIKRTV
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

anti-CD29-1-Fab sequences:

9-1 HC (SEQ ID NO: 20):

QVQLQQPGTELVRPGSSVKLSCKASGYTFTSYWVNWVKQRPDQGLEWIGRIDPYDS
ETHYNQKFTDKAISTIDTSSNTAYMQLSTLTSDASAVYYCSRSPRDSSTNLADWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTC

9-1 LC (SEQ ID NO: 21):

DIVMTQSPATLSVTPGDRVSLSCRASQSISDYLHWYQQKSHESPRLLIKYASQSISGIPS
RFSGSGSGSDFTLSINSVEPEDVGVYYCQNGHSFPLTFGAGTKLELRRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

mutOKT8-Fab sequences:

mutOKT8 HC (SEQ ID NO: 22):

QVQLVQSGAEDKKPGASVKVSCKASGFNIKDTYIHWVRQAPGQGLEWMGRIDPAND
NTLYASKFQGRVTITADTSSNTAYMELSSLRSEDTAVYYCGRGAGAYVFDHWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTC

mutOKT8 LC (SEQ ID NO: 23):

DIVMTQSPSSLSASVGDRVTITCRTSRSISAALAWYQEKPGKAPKLLIYSGSTLQSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQHNENPLTFGQGTKVEIKRTVAAPSVFIFP

PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES.

Anti-CD56 A1 Fab sequence

A1 bDS HC (SEQ ID NO: 26):

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSNWIRQSPSGLEWL
GRTYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCARENIAA

WTWAFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT
VSWNSGALTSGVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSSDKTHTCGGHHHHHH

A1 bDS LC (SEQ ID NO: 27):

EIVMTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGLAPRLLIYDTSLRATDI
PDRFSGSGSGTAFTLTISRLEPEDFAVYYCQQYGSSPTFGQGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD56 A2 Fab sequence

A2 bDS HC (SEQ ID NO: 28):

EVQLVQSGAEVKKPGSSVKVSCKASGGTFTGYYMHWVRQAPGQGLEWMGWINPN
SGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARDLSSGYSGYFDY
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSD
KTHTCGGHHHHHH

A2 bDS LC (SEQ ID NO: 29):

DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLNWYLQKPGQSPQLLIYLGSN
RASGVPDRFSGSGSGTDFTLKISRVEGEDVGDYYCMQALQSPFTFGQGTKLEIKRTV

AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD56 A3 Fab sequence

A3 bDS HC (SEQ ID NO: 30):

EVQLVQSGAEVKKPGSSVKVSCKASGGTFTGYYMHWVRQAPGQGLEWMGWINPN
SGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARDLSSGYSGYFDY
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSD
KTHTCGGHHHHHH

A3 bDS LC (SEQ ID NO: 31):

DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNFLDWYLQKPGQSPQLLIYLGSN
RASGVPDRFSGSGSGTDFTLKISRVEADDVGVYYCMQSLQTPWTFGHGTKVEIKRTV
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD56 Lorvotuzumab Fab sequence

Lorvotuzumab bDS HC (SEQ ID NO: 32):

QVQLVESGGG VVQPGRSLRL SCAASGFTFS SFGMHWVRQA
PGKGLEWVAYISSGSFTIYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED
TAVYYCARMR KGYAMDYWGQ
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKT
HTCHHHHHH

Lorvotuzumab bDS LC (SEQ ID NO: 33):

DVVMTQSPLSLPVTLGQPASISCRSSQIIIHSDGNTYLEWFQQRPGQSPRRLIYKVSNR
FSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPHTFGQGTKVEIKRTVA

APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD2 RPA-2.10v1 Fab sequence

RPA-2.10v1 bDS HC (SEQ ID NO: 34):

EVKLVESGGGLVKPGGSLKLSCAASGFTFSSYDMSWVRQTPEKRLEWVASISGGGFL
YYLDSVKGRFTISRDNARNILYLHMTSLRSEDTAMYYCARSSYGEIMDYWGQGTSV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTCP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTCHH
HHHH

RPA-2.10v1 bDS LC (SEQ ID NO: 35):

DILLTQSPAILSVSPGERVSFSCRASQRIGTSIHWYQQRTTGSPRLLIKYASESISGIPSR
FSGSGSGTDFTLSINSVESEDVADYYCQQSHGWPFTFGGGTKLEIERTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLC
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD1374B4-1 Fab sequence

4B4-1 bDS HC (SEQ ID NO: 36):

QVQLQQPGAELVKPGASVKLSCKASGYTFSSYWMHWVKQRPGQVLEWIGEINPGN
GHTNYNEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARSFTTARGFAYWGQ
GTLVTVSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHT
CHHHHHH

4B4-1 bDS LC (SEQ ID NO: 37):

DIVMTQSPATQSVTPGDRVSLSCRASQTISDYLHWYQQKSHESPRLLIKYASQSISGIP
SRFSGSGSGSDFTLSINSVEPEDVGVYYCQDGHSFPPTFGGGTKLEIKRTVAAPSVFIF

PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

hSP34-hlam NoDS HC (SEQ ID NO: 38):

EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKY
NNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISY
WAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSSDKTHTC

hSP34-hlam NoDS LC (SEQ ID NO: 39):

QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLA
PGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLSQPK
AAPSVTLFPPSSEELQANKATLVCLVSDFYPGAVTVAWKADGSPVKVGVETTKPSK
QSNNKYAASSYLSLTPEQWKSHRSYSCRVTHEGSTVEKTVAPAESS

hSP34-hlam DS HC (SEQ ID NO: 40):

EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKY
NNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISY
WAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSCDKTHTC

hSP34-hlam DS LC (SEQ ID NO: 41):

QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLA
PGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLSQPK
AAPSVTLFPPSSEELQANKATLVCLVSDFYPGAVTVAWKADGSPVKVGVETTKPSK
QSNNKYAASSYLSLTPEQWKSHRSYSCRVTHEGSTVEKTVAPAECS

Anti-CD2 TS2/18.1 DS Fab

TS2/18.1 DS HC (SEQ ID NO: 42):

EVQLVESGGGLVMPGGSLKLSCAASGFAFSSYDMSWVRQTPEKRLEWVAYISGGGF
TYYPDTVKGRFTLSRDNAKNTLYLQMSSLKSEDTAMYYCARQGANWELVYWGQG
TLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC

TS2/18.1 DS LC (SEQ ID NO: 43):

DIVMTQSPATLSVTPGDRVFLSCRASQSISDFLHWYQQKSHESPRLLIKYASQSISGIPS
RFSGSGSGSDFTLSINSVEPEDVGVYFCQNGHNFPPTFGGGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Anti-CD29.6 DS Fab

9.6 DS HC (SEQ ID NO: 44):

QVQLQQPGAELVRPGSSVKLSCKASGYTFTRYWIHWVKQRPIQGLEWIGNIDPSDSE
THYNQKFKDKATLTVDKSSGTAYMQLSSLTSEDSAVYYCATEDLYYAMEYWGQGT
SVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC

9.6 DS LC (SEQ ID NO: 45):

NIMMTQSPSSLAVSAGEKVTMTCKSSQSVLYSSNQKNYLAWYQQKPGQSPKLLIYW
ASTRESGVPDRFTGSGSGTDFTLTISSVQPEDLAVYYCHQYLSSHTFGGGTKLEIKRTV
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

hSP34-hlam bDS HC (SEQ ID NO: 46):

EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKY
NNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISY
WAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN

SGALTSGVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSSDKTHTCHHHHHH

hSP34-hlam bDS LC (SEQ ID NO: 47):

QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLA
PGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLSQPK
AAPSVTLFPPSSEELQANKATLVCLVSDFYPGAVTVAWKADGSPVKVGVETTKPSK
QSNNKYAACSYLSLTPEQWKSHRSYSCRVTHEGSTVEKTVAPAESS

Anti-CD3 TR66 bDS Fab sequence

TR66 bDS HC (SEQ ID NO: 48):

QVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSR
GYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDNYSLDYWG
QGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKT
HTCHHHHHH

TR66 bDS LC (SEQ ID NO: 49):

QIVLTQSPSSLSASLGEKVTMTCRASSSVSYMNWYQQKPGTSPKRWIYDTSKVASGV
PDRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD3 TRX4 bDS Fab sequence

TRX4 bDS HC (SEQ ID NO: 50):

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTSGGR
TYYRDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDYWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV

HTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHT
CHHHHHH

TRX4 bDS LC (SEQ ID NO: 51):

DIQLTQPNSVSTSLGSTVKLSCTLSSGNIENNYVHWYQLYEGRSPTTMIYDDDKRPD
GVPDRFSGSIDRSSNSAFLTIHNVAIEDEAIYFCHSYVSSFNVFGGGTKLTVLGQPKAN
PTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSN
NKYAACSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTESS

Anti-CD3 HzUCHT1 bDS Fab sequence

HzUCHT1(Y59T) bDS HC (SEQ ID NO: 52):

EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINPTK
GVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFD
VWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA
LTSGVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSS
DKTHTCHHHHHH

HzUCHT1 bDS LC (SEQ ID NO: 53):

DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGV
PSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD3 Teplizumab bDS Fab sequence

Teplizumab bDS HC (SEQ ID NO: 54):

QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRG
YTNYNQKVKDRFTISRDNSKNTAFLQMDSLRPEDTGVYFCARYYDDHYCLDYWGQ
GTPVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV

HTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHT
CHHHHHH

Teplizumab bDS LC (SEQ ID NO: 55):

DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGV
PSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFGQGTKLQITRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD8 TRX2 bDS Fab sequence

TRX2 bDS HC (SEQ ID NO: 56):

QVQLVESGGGVVQPGRSLRLSCAASGFTFSDFGMNWVRQAPGKGLEWVALIYYDG
SNKFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPHYDGYYHFFDS
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSD
KTHTC

TRX2 bDS LC (SEQ ID NO: 57):

DIQMTQSPSSLSASVGDRVTITCKGSQDINNYLAWYQQKPGKAPKLLIYNTDILHTG
VPSRFSGSGSGTDFTFTISSLQPEDIATYYCYQYNNGYTFGQGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD2 Lo-CD2b bDS Fab sequence

Lo-CD2b bDS HC (SEQ ID NO: 58):

EVQLVESGGGLVQPGASLKLSCVASGFTFSDYWMSWVRQTPGKPMEWIGHIKYDGS
YTNYAPSLKNRFTISRDNAKTTLYLQMSNVRSEDSATYYCAREAPGAASYWGQGTL
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
CPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTC

Lo-CD2b bDS LC (SEQ ID NO: 59):

DVVLTQTPVAQPVTLGDQASISCRSSQSLVHSNGNTYLEWFLQKPGQSPQLLIYKVS
NRFSGVPDRFIGSGSGSDFTLKISRVEPEDWGVYYCFQGTHDPYTFGAGTKLELKRT
VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD235.1 bDS Fab sequence

35.1 bDS HC (SEQ ID NO: 60):

EVQLQQSGAELVKPGASVKLSCRTSGFNIKDTYIHWVKQRPEQGLKWIGRIDPANGN
TKYDPKFQDKATVTADTSSNTAYLQLSSLTSEDTAVYYCVTYAYDGNWYFDVWGA
GTAVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKT
HTC

35.1 bDS LC (SEQ ID NO: 61):

DIKMTQSPSSMYVSLGERVTITCKASQDINSFLSWFQQKPGKSPKTLIYRANRLVDGV
PSRFSGSGSGQDYSLTISSLEYEDMEIYYCLQYDEFPYTFGGGTKLEMKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD2 OKT11 bDS Fab sequence

OKT11 bDS HC (SEQ ID NO: 62):

QVQLQQPGAELVRPGTSVKLSCKASGYTFTSYWMHWIKQRPEQGLEWIGRIDPYDS
ETHYNEKFKDKAILSVDKSSSTAYIQLSSLTSDDSAVYYCSRRDAKYDGYALDYWGQG
TSVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTC
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTC

OKT11 bDS LC (SEQ ID NO: 63):

DIVMTQAAPSVPVTPGESVSISCRSSKTLLHSNGNTYLYWFLQRPGQSPQVLIYRMSN
LASGVPNRFSGSGSETTFTLRISRVEAEDVGIYYCMQHLEYPYTFGGGTKLEIERTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD11a HzMHM24 bDS Fab sequence

HzMHM24 bDS HC (SEQ ID NO: 64):

EVQLVESGGGLVQPGGSLRLSCAASGYSFTGHWMNWVRQAPGKGLEWVGMIHPSD
SETRYNQKFKDRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARGIYFYGTTYFDYW
GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSD
KTHTCHHHHHH

HzMHM24 bDS LC (SEQ ID NO: 65):

DIQMTQSPSSLSASVGDRVTITCRASKTISKYLAWYQQKPGKAPKLLIYSGSTLQSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQHNEYPLTFGQGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD18 h1B4 bDS Fab sequence

h1B4 bDS HC (SEQ ID NO: 66):

EVQLVESGGDLVQPGRSLRLSCAASGFTFSDYYMSWVRQAPGKGLEWVAAIDNDG
GSISYPDTVKGRFTISRDNAKNSLYLQMNSLRVEDTALYYCARQGRLRRDYFDYWG
QGTLVTVSTASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDK
THTCHHHHHH

h1B4 bDS LC (SEQ ID NO: 67):

DIQMTQSPSSLSASVGDRVTITCRASESVDSYGNSFMHWYQQKPGKAPKLLIYRASN
LESGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQSNEDPLTFGQGTKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYSLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD18 Erlizumab bDS Fab sequence

Erlizumab bDS HC (SEQ ID NO: 68):

EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPKN
GGTSHNQRFMDRFTISVDKSTSTAYMQMNSLRAEDTAVYYCARWRGLNYGFDVRY
FDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSSDKTHTCHHHHHH

Erlizumab bDS LC (SEQ ID NO: 69):

DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSG
VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD4/CD8 Ibalizumab/TRX2 bDS Fab-ScFv sequence

Ibalizumab/TRX2 bDS Fab-ScFv HC (SEQ ID NO: 70):

QVQLQQSGPEVVKPGASVKMSCKASGYTFTSYVIHWVRQKPGQGLDWIGYINPYND
GTDYDEKFKGKATLTSDTSTSTAYMELSSLRSEDTAVYYCAREKDNYATGAWFAY
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSD
KTHTCHHHHHH

Ibalizumab/TRX2 bDS Fab-ScFv LC (SEQ ID NO: 71):

DIVMTQSPDSLAVSLGERVTMNCKSSQSLLYSTNQKNYLAWYQQKPGQSPKLLIYW
ASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQYYSYRTFGGGTKLEIKRT
VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGESGGGGSGGG
GSGGGGSQVQLVESGGGVVQPGRSLRLSCAASGFTFSDFGMNWVRQAPGKGLEWV
ALIYYDGSNKFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPHYDGY
YHFFDSWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRV
TITCKGSQDINNYLAWYQQKPGKAPKLLIYNTDILHTGVPSRFSGSGSGTDFTFTISSL
QPEDIATYYCYQYNNGYTFGQGTKVEIK

Anti-CD4 Ibalizumab NoDS Fab sequence

Ibalizumab NoDS LC (SEQ ID NO: 72):

QVQLQQSGPEVVKPGASVKMSCKASGYTFTSYVIHWVRQKPGQGLDWIGYINPYND
GTDYDEKFKGKATLTSDTSTSTAYMELSSLRSEDTAVYYCAREKDNYATGAWFAY
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSD
KTHTC

Ibalizumab NoDS HC (SEQ ID NO: 73):

DIVMTQSPDSLAVSLGERVTMNCKSSQSLLYSTNQKNYLAWYQQKPGQSPKLLIYW
ASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQYYSYRTFGGGTKLEIKRT
VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD4 OKT4 bDS Fab sequence

OKT4 bDS LC (SEQ ID NO: 74):

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKRLEWVSAISDHST
NTYYPDSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCARKYGGDYDPFDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS

GVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDK
THTCHHHHHH

OKT4 bDS HC (SEQ ID NO: 75):

DIQMTQSPSSLSASVGDRVTITCQASQDINNYIAWYQHKPGKGPKLLIHYTSTLQPGIP
SRFSGSGSGRDYTLTISSLQPEDFATYYCLQYDNLLFTFGGGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD4 T023200008 Nb sequence (SEQ ID NO: 76)

CDR1, CDR2, CDR3 underlined based on IMGT designation:

EVQLVESGGGSVQPGGSLTLSCGTSGRTFNVMGWFRQAPGKEREFVAAVRWSSTGI
YYTQYADSVKSRFTISRDNAKNTVYLEMNSLKPEDTAVYYCAADTYNSNPARWDG
YDFRGQGTLVTVSSGGCGGHHHHHH

Anti-CD8 BDSn Nb sequence (SEQ ID NO: 77)

CDR1, CDR2, CDR3 underlined based on IMGT designation:

EVQLVESGGGLVQAGGSLRLSCAASGSTFSDYGVGWFRQAPGKGREFVADIDWNGE
HTSYADSVKGRFATSRDNAKNTAYLQMNSLKPEDTAVYYCAADALPYTVRKYNYW
GQGTQVTVSSGGCGGHHHHHH

Anti-CD3 T0170117G03-A Nb sequence (SEQ ID NO: 78)
EVQLVESGGGPVQAGGSLRLSCAASGRTYRGYSMGWFRQAPGKEREFVAAIVWSG
GNTYYEDSVKGRFTISRDNAKNIMYLQMTSLKPEDSATYYCAAKIRPYIFKIAGQYD
YWGQGTLVTVSSAGGGSGGHHHHHHC

Anti-CD3 T0170060E11 Nb sequence (SEQ ID NO: 79)
EVQLVESGGGLVQPGGSLRLSCAASGDIYKSFDMGWYRQAPGKQRDLVAVIGSRGN
NRGRTNYADSVKGRFTISRDGTGNTVYLLMNKLRPEDTAIYYCNTAPLVAGRPWGR
GTLVTVSSGGGSGGHHHHHHC

Anti-CD7 V1 Nb sequence (SEQ ID NO: 80)

DVQLQESGGGLVQAGGSLRLSCAVSGYPYSSYCMGWFRQAPGKEREGVAAIDSDG
RTRYADSVKGRFTISQDNAKNTLYLQMNRMKPEDTAMYYCAARFGPMGCVDLSTL
SFGHWGQGTQVTVSITGGGCHHHHHHHH

Anti-TCR T017000700 Nb sequence (SEQ ID NO: 81)

CDR1, CDR2, CDR3 underlined based on IMGT designation:

EVQLVESGGGVVQPGGSLRLSCVASGYVHKINFYGWYRQAPGKEREKVAHISIGDQ
TDYADSAKGRFTISRDESKNTVYLQMNSLRPEDTAAYYCRALSRIWPYDYWGQGTL
VTVSSGGCGGHHHHHH

Anti-CD2828CD065G01 Nb sequence (SEQ ID NO: 82)

EVQLVESGGGLVQPGGSLRLSCAASGSIFRLHTMEWYRRTPETQREWVATITSGGTT
NYPDSVKGRFTISRDDTKKTVYLQMNSLKPEDTAVYYCHAVATEDAGFPPSNYWG
QGTLVTVSSGGCGGHHHHHH

Anti-CD3 T0170061C09 Nb sequence (SEQ ID NO: 83)

EVQLVESGGGPVQAGGSLRLSCAASGRTYRGYSMGWFRQAPGREREFVAAIVWSD
GNTYYEDSVKGRFTISRDNAKNTMYLQMTSLKPEDSATYYCAAKIRPYIFKIAGQYD
YWGQGTLVTVSSGGCGGHHHHHH

Anti-CD312D2 bDS Fab sequence

12D2 bDS HC (SEQ ID NO: 84):

EVKLVESGGGLVQPGRSLRLSCAASGFNFYAYWMGWVRQAPGKGLEWIGEIKKDG
TTINYTPSLKDRFTISRDNAQNTLYLQMTKLGSEDTALYYCAREERDGYFDYWGQG
VMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHT
CGGHHHHHH

12D2 bDS LC (SEQ ID NO: 85):

QFVLTQPNSVSTNLGSTVKLSCKRSTGNIGSNYVNWYQQHEGRSPTTMIYRDDKRPD
GVPDRFSGSIDRSSNSALLTINNVQTEDEADYFCQSYSSGIVFGGGTKLTVLSQPKAA
PSVTLFPPSSEELQANKATLVCLVSDFYPGAVTVAWKADGSPVKVGVETTKPSKQSN
NKYAACSYLSLTPEQWKSHRSYSCRVTHEGSTVEKTVAPAESS

Anti-CD288G8A Fab sequence

8G8A bDS HC (SEQ ID NO: 86):

EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVIQWVKQKPGQGLEWIGSINPYND
YTKYNEKFKGKATLTSDKSSITAYMEFSLTSEDSALYCARWGDGNYWGRGTLTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTCPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTCGGHHH
HHH

8G8A bDS LC (SEQ ID NO: 87):

DIEMTQSPAIMSASLGERVTMTCTASSSVSSSYFHWYQKPGSSPKLCIYSTSNLASGV
PPRFSGSGSTSYSLTISMEAEDAATYFCHQYHRSPTFGGGTKLETKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLC
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD282E12 Fab sequence

2E12 bDS HC (SEQ ID NO: 88):

QVQLKESGPGLVAPSQSLSITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGS
TDYNSALKSRLSITKDNSKSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYW
GQGTSVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDK
THTCGGHHHHHH

2E12 bDS LC (SEQ ID NO: 89):

DIVLTQSPASLAVSLGQRATISCRASESVEYYVTSLMQWYQQKPGQPPKLLISAASNV
ESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQQSRKVPWTFGGGTKLEIKRRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD28 CD28.9.3 Fab sequence

CD28.9.3 bDS HC (SEQ ID NO: 90):

QVKLQQSGPGLVTPSQSLSITCTVSGFSLSDYGVHWVRQSPGQGLEWLGVIWAGGG
TNYNSALMSRKSISKDNSKSQVFLKMNSLQADDTAVYYCARDKGYSYYYSMDYW

GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSD
KTHTCGGHHHHHH

CD28.9.3 bDS LC (SEQ ID NO: 91):

DIVLTQSPAS LAVSLGQRAT ISCRASESVEYYVTSLMQWY QQKPGQPPKL
LIFAASNVES GVPARFSGSG SGTNFSLNIHPVDEDDVAMY FCQQSRKVPY
TFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGES

Anti-CD28 HzTN228 Fab sequence

HzTN228 bDS HC (SEQ ID NO: 92):

QVQLQESGPGLVKPSETLSLTCAVSGFSLTSYGVHWIRQPGKGLEWLGVIWPGTNFN
SALMSRLTISEDTSKNQVSLKLSSVTAADTAVYYCARDRAYGNYLYAMDYWGQGTL
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
CPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTC
GGHHHHHH

HzTN228 bDS LC (SEQ ID NO: 93):

DIQMTQSPSLSASVGDRVTITCRASESVEYVTSLMQWYQKPGKAPKLLIYAASNVDS
GVPSRFSGSGTDFTLTISLQPEDIATYCQSRKVPFTFGGGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLCS
TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD28 TGN2122.C Fab sequence

TGN2122.C bDS HC (SEQ ID NO: 94):

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYKIHWVRQAPGQGLEWIGYIYPYSG
SSDYNQKFKSRATLTVDNSISTAYMELSRLRSDDTAVYYCARGGDAMDYWGQGTL
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
CPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTC
GGHHHHHH

TGN2122.C bDS LC (SEQ ID NO: 95):

DIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQRKPGKAPKLLIYGATNLADG
VPSRFSGSGSGRDYTLTISSLQPEDFATYFCQNILGTWTFGGGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD28 TGN2122.H Fab sequence

TGN2122.H bDS HC (SEQ ID NO: 96):

EVQLVESGGGLVQPGGSLRLSCAASGFTFNIYYMSWVRQAPGKGLELVAAINPDGG
NTYYPDTVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARYGGPGFDSWGQGT
LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTC
GGHHHHHH

TGN2122.H bDS LC (SEQ ID NO: 97):

ENVLTQSPATLSLSPGERATLSCSASSSVSYMHWYQQKPGQAPRLWIYDTSKLASGIP
ARFSGSGSRNDYTLTISSLEPEDFAVYYCFPGSGFPFMYTFGGGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLCSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGES

Anti-CD8 TRX2 ScFv sequence (SEQ ID NO: 98):

QVQLVESGGGVVQPGRSLRLSCAASGFTFSDFGMNWVRQAPGKGLEWVALIYYDG
SNKFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPHYDGYYHFFDS
WGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCKG
SQDINNYLAWYQQKPGKAPKLLIYNTDILHTGVPSRFSGSGSGTDFTFTISSLQPEDIA
TYYCYQYNNGYTFGQGTKVEIKGGGSGGCGGHHHHHH

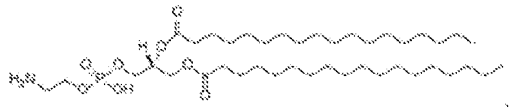
V1 VHH-CH1 bDS HC (SEQ ID NO: 99):

DVQLQESGGGLVQAGGSLRLSCAVSGYPYSSYCMGWFRQAPGKEREGVAAIDSDG
RTRYADSVKGRFTISQDNAKNTLYLQMNRMKPEDTAMYYCAARFGPMGCVDLSTL
SFGHWGQGTQVTVSITASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTCPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSSDKTHTCGGHHHHHH

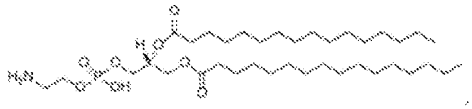
In some embodiments, the targeting moiety comprises a polypeptide sequence as disclosed herein. In some embodiments, the targeting moiety comprises all six CDRs of a polypeptide sequence as disclosed herein. In some embodiments, the targeting moiety comprises CDR1, CDR2, and CDR3 of an immunoglobulin single variable domain (ISVD) as disclosed herein. In further embodiments, the targeting moiety binds to the same epitope on the targeting molecule that a polypeptide sequence as disclosed herein binds to. In further embodiments, the targeting moiety competes with a polypeptide sequence as disclosed herein to bind to the same epitope on the targeting molecule.

In certain embodiments, the targeting group or immune cell targeting group (e.g., T cell-targeting agent, B cell-targeting agent, or NK-cell targeting agent) may be covalently coupled to a lipid via a polyethylene glycol (PEG) containing linker.

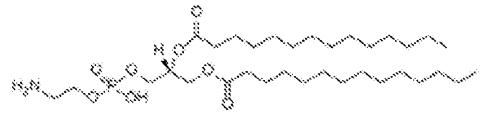
In other embodiments, the lipid used to create a conjugate may be selected from distearoyl-phosphatidylethanolamine (DSPE):



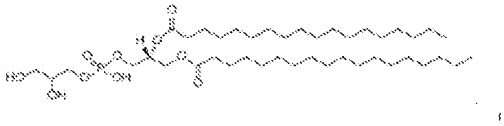
dipalmitoyl-phosphatidylethanolamine (DPPE):



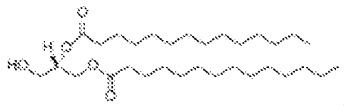
dimyrstoyl-phosphatidylethanolamine (DMPE):



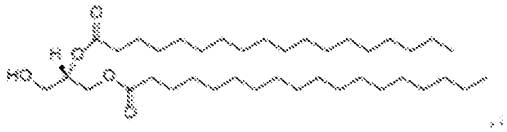
distearoyl-glycero-phosphoglycerol (DSPG):



dimyristoyl-glycerol (DMG):

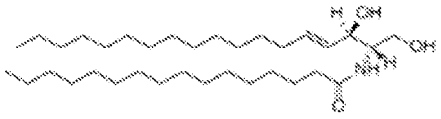


distearoylglycerol (DSG):



and

N-palmitoyl-sphingosine (C16-ceramide)



The immune cell targeting group can be covalently coupled to a lipid either directly or via a linker, for example, a polyethylene glycol (PEG) containing linker. In certain embodiments, the PEG is PEG 1000, PEG 2000, PEG 3400, PEG 3000, PEG 3450, PEG 4000, or PEG 5000. In certain, embodiments, the PEG is PEG 2000.

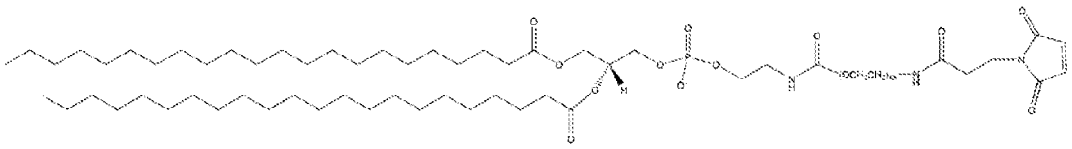
In some embodiments, the lipid-immune cell targeting group conjugate is present in the lipid blend in a range of 0.001-0.5 mole percent, 0.001-0.3 mole percent, 0.002-0.2 mole percent, 0.01-0.1 mole percent, 0.1-0.3 mole percent, or 0.1-0.2 mole percent. The lipid-immune cell targeting group conjugate may be present in the lipid blend in a range of 0.001-0.5 mol percent, 0.001-0.1 mole percent, 0.01-0.5 mole percent, 0.05-0.5 mole percent, 0.1-0.5 mole percent, 0.1-0.3 mole percent, 0.1-0.2 mole percent, 0.2-0.3 mole percent, of about 0.01 mole percent, about 0.05 mole percent, about 0.1 mole percent, about 0.15 mole percent, about 0.2 mole percent, about 0.25 mole percent, about 0.3 mole percent, about 0.35 mole percent, about 0.4 mole percent, about 0.45 mole percent, or about 0.5 mole percent.

In certain embodiments, the lipid immune-cell targeting agent conjugate comprises DSPE, a PEG component and a targeting antibody. In certain embodiments, the antibody is a T-cell targeting agent, for example, an anti-CD2 antibody, an anti-CD3 antibody, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD7 antibody, an anti CD8 antibody, or an anti-TCR β antibody.

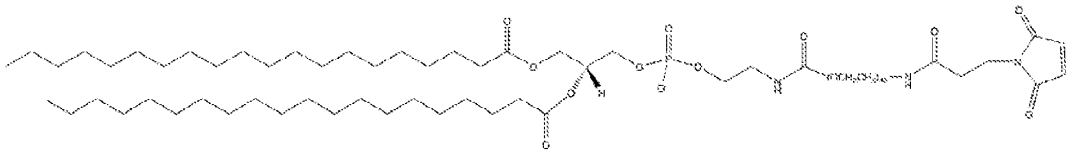
An exemplary lipid-immune cell targeting group conjugate comprises DSPE and PEG 2000, for example, as described in Nellis et al. (2005) BIOTECHNOL. PROG.21, 205-220. An exemplary conjugate comprises the structure of Formula (III), where the scFv represents an engineered antibody binding site that binds to a target of interest. In certain embodiments, the engineered antibody binding site binds to any of the targets described hereinabove. In certain embodiments, the engineered antibody binding site can be, for example, an engineered anti-CD3 antibody or an engineered anti-CD8 antibody. In certain embodiments, the engineered antibody binding site can be, for example, an engineered anti-CD2 antibody or an engineered anti-CD7 antibody.

In certain embodiments, the lipid immune-cell targeting agent conjugate comprises DBPE or DAPE, a PEG component and a targeting antibody. In certain embodiments, the antibody is a T-cell targeting agent, for example, an anti-CD2 antibody, an anti-CD3 antibody, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD7 antibody, an anti CD8 antibody, or an anti-TCR β antibody.

In some embodiments, the lipid immune-cell targeting agent conjugate comprises DBPE or DAPE, a PEG component represented as:

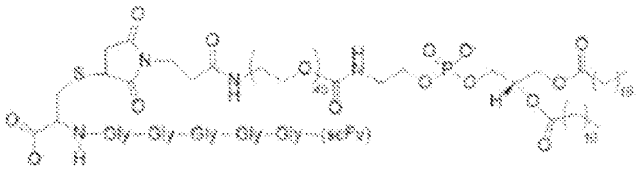


DBPE-PEG2000-Mal;



DAPE-PEG2000-Mal.

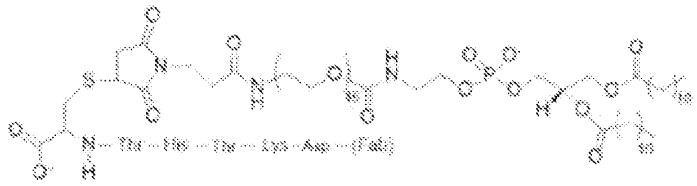
An example of a compound of Formula (III) (SEQ ID NO: 160) is as shown below:



(III).

It is contemplated that the scFv in Formula (III) may be replaced with an intact antibody or an antigen fragment thereof (e.g., a Fab).

Another example of a compound of Formula (IV) (SEQ ID NO: 161) is as shown below:

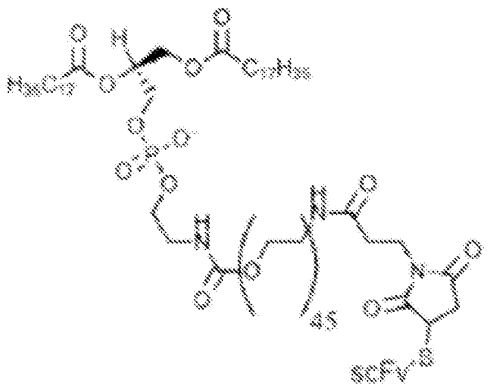


the production of which is described in Nellis et al. (2005) supra, or U.S. Patent No.7,022,336. It is contemplated that the Fab in Formula (IV) may be replaced with an intact antibody or an antigen fragment thereof (e.g., an $(Fab')_2$ fragment) or an engineering antibody binding site (e.g., an scFv).

Other lipid immune cell target group conjugates are described, for example, in U.S. Patent No. 7,022,336, where the targeting group may be replaced with a targeting group of interest, for example, a targeting group that binds an T-cell or NK cell surface antigen as described hereinabove.

In certain embodiments, the lipid component of an exemplary conjugate of Formula (II) can be any of the lipids described herein. In some embodiments, the lipid component of a conjugate of Formula (II) is based on an ionizable, cationic lipid described herein, for example, an ionizable, cationic lipid of Formula (A-I), (A-II), (B-I), (B-II), (C-I), (C-II), (C-III), (C-IV), (D-I), (D-II), (E-1), or (E-II), or a salt thereof . For example, an exemplary ionizable, cationic lipid can be selected from any of Tables A, B, C, D, or E or a salt thereof.

In certain embodiments, the conjugate based on a lipid of the present disclosure may include:



, where scFv represents an engineered antibody binding site that binds a target described hereinabove, e.g., CD2, CD3, CD7, or CD8.

In certain embodiments, the lipid blend may further comprise free PEG-lipid, e.g., so as to reduce the amount of non-specific binding via the targeting group. The free PEG-lipid can be the same or different from the PEG-lipid included in the conjugate. In certain embodiments, the free PEG-lipid is selected from the group consisting of PEG-distearoyl-phosphatidylethanolamine (PEG-DSPE) or PEG-dimyrstoyl-phosphatidylethanolamine (PEG-DMPE), N-(Methylpolyoxyethylene oxycarbonyl)-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE-PEG) 1,2-Dimyristoyl-rac-glycero-3-methylpolyoxyethylene (PEG-DMG), 1,2-Dipalmitoyl-rac-glycero-3-methylpolyoxyethylene (PEG-DPG), 1,2-Dioleoyl-rac-glycerol, methoxypolyethylene Glycol (DOG-PEG) 1,2-Distearoyl-rac-glycero-3- methylpolyoxyethylene (PEG-DSG), N-palmitoyl-sphingosine-1- {succinyl[methoxy(polyethylene glycol)] (PEG-ceramide), DSPE-PEG-cysteine, or a derivative thereof. In some embodiments, the average PEG lengths are between 2000-5000, with 2000, 3400, or 5000. A final composition may contain a mixture of two or more of these pegylated lipids. In certain embodiments, the LNP composition comprises a mixture of PEG-lipids with myristoyl and stearic acyl chains. In certain embodiments, the LNP composition comprises a mixture of PEG- lipids with palmitoyl and stearoyl acyl chains.

In certain embodiments, the derivative of the PEG-lipid has a methyoxy, hydroxyl or a carboxylic acid end group at the PEG terminus.

The lipid-immune cell targeting group conjugate can be incorporated into LNPs as described below, for example, in LNPs containing, for example, an ionizable cationic lipid, a sterol, a neutral phospholipid and a PEG-lipid. It is contemplated that, in certain embodiments, the LNPs containing the lipid-immune cell targeting group can contain an ionizable cationic lipid described herein or a cationic lipid described, for example, in U.S. Patent Nos.10,221,127, 10,653,780 or U.S. Published application Nos. US2018/0085474, US2016/0317676, International Publication No. WO2009/086558, or Miao et al. (2019) NATURE BIOTECH 37:1174-1185, or Jayaraman et al. (2012) ANGEW CHEM INT.51: 8529-8533.

The LNPs can be formulated using the methods and other components described below in the following sections.

IV. LIPID NANOPARTICLE COMPOSITIONS

The invention provides a lipid nanoparticle (LNP) composition comprising a lipid blend that contains an ionizable cationic lipid described herein and/or a lipid-immune cell targeting agent conjugate described herein. In certain embodiments, the lipid blend may comprise an ionizable, cationic lipid described herein and one or more of a sterol, a neutral phospholipid, a PEG-lipid, and a lipid-immune cell targeting group conjugate.

In certain embodiments, the ionizable, cationic lipid described herein may be present in the lipid blend in a range of 30-70 mole percent, 30-60 mole percent 30-50 mole percent, 40-70 mole percent, 40-60 mole percent, 40-50 mole percent, 50-70 mole percent, 50-60 mole percent, or of about 30 mole percent, about 35 mole percent, about 40 mole percent, about 45 mole percent, about 50 mole percent, about 55 mole percent, about 60 mole percent, about 65 mole percent, or about 70 mole percent.

STEROL

In certain embodiments, the lipid blend of the lipid nanoparticle may comprise a sterol component, for example, one or more sterols selected from the group consisting of cholesterol, fecosterol, β-sitosterol, ergosterol, campesterol, stigmasterol, stigmastanol, and brassicasterol. In certain embodiments, the sterol is cholesterol.

The sterol (e.g., cholesterol) may be present in the lipid blend in a range of 20-70 mole percent, 20-60 mole percent, 20-50 mole percent, 30-70 mole percent, 30-60 mole percent, 30-50 mole

percent, 40-70 mole percent, 40-60 mole percent, 40-50 mole percent, 50-70 mole percent, 50-60 mole percent, or about 20 mole percent, about 25 mole percent, about 30 mole percent, about 35 mole percent, about 40 mole percent, about 45 mole percent, about 50 mole percent, about 55 mole percent, about 60 mole percent or about 65 mole percent.

In some embodiments, the sterol is present in the lipid blend in a range of 30-50 mole percent. In some embodiments, the sterol is present in the lipid blend in a range of 20-70 mole percent.

NEUTRAL PHOSPHOLIPID

In certain embodiments, the lipid blend of the lipid nanoparticle may contain one or more neutral phospholipids. The neutral phospholipid can be selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), hydrogenated soy phosphatidylcholine (HSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and sphingomyelin (SM).

Other neutral phospholipids can be selected from the group consisting of distearoyl-phosphatidylethanolamine (DSPE), dimyrstoyl-phosphatidylethanolamine (DMPE), distearoyl-glycero-phosphocholine (DSPC), hydrogenated soy phosphatidylcholine (HSPC), dioleoyl-glycero-phosphoethanolamine (DOPE), dilinoleoyl-glycero-phosphocholine (DLPC), dimyristoyl-glycero-phosphocholine (DMPC), dioleoyl-glycero-phosphocholine (DOPC), dipalmitoyl-glycero-phosphocholine (DPPC), diundecanoyl-glycero-phosphocholine (DUPC), palmitoyl-oleoyl-glycero-phosphocholine (POPC), dioctadecenyl-glycero-phosphocholine, oleoyl-cholesterylhemisuccinoyl-glycero-phosphocholine, hexadecyl-glycero-phosphocholine, dilinolenoyl-glycero-phosphocholine, diarachidonoyl-glycero-3-phosphocholine, didocosahexaenoyl-glycero-phosphocholine, and sphingomyelin.

The neutral phospholipid may be present in the lipid blend in a range of 1-10 mole percent, 1-15 mole percent, 1-12 mole percent, 1-10 mole percent, 3-15 mole percent, 3-12 mole percent, 3-10 mole percent, 4-15 mole percent, 4-12 mole percent, 4-10 mole percent, 4-8 mole percent, 5-15 mole percent, 5-12 mole percent, 5-10 mole percent, 6-15 mole percent, 6-12 mole percent, 6-10 more percent, or about 1 mole percent, about 2 mole percent, about 3 mole percent, about 4 mole percent, about 5 mole percent, about 6 mole percent, about 7 mole percent, about 8 mole percent,

about 9 mole percent, about 10 mole percent, about 11 mole percent, about 12 mole percent, about 13 mole percent, about 14 mole percent, or about 15 mole percent.

In some embodiments, the neutral phospholipid is present in the lipid blend in a range of 1-15 mole percent, such as about 5-15 mole percent, or about 5 to 10 mole percent.

PEG-LIPID

The lipid blend of the lipid nanoparticle may include one or more PEG or PEG-modified lipids. Such species may be alternately referred to as PEGylated lipids. A PEG lipid is a lipid modified with polyethylene glycol. As noted above, free PEG-lipids can be included in the lipid blend to reduce or eliminate non-specific binding via a targeting group when a lipid-immune cell targeting group is included in the lipid blend. In some embodiments the free PEG-lipid is a mixture of two or more unique free PEG-lipids.

A PEG lipid (e.g., a free PEG-lipid) may be selected from the non-limiting group consisting of PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, and PEG-modified dialkylglycerols. For example, a PEG lipid may be PEG- dioleoylgylcerol (PEG-DOG), PEG-dimyristoyl-glycerol (PEG-DMG), PEG-dipalmitoyl-glycerol (PEG-DPG), PEG-dilinoleoyl-glycero-phosphatidyl ethanolamine (PEG-DLPE), PEG-dimyrstoyl-phosphatidylethanolamine (PEG-DMPE), PEG-dipalmitoyl- phosphatidylethanolamine (PEG-DPPE), PEG-distearoylglycerol (PEG-DSG), PEG-diacylglycerol (PEG-DAG, e.g., PEG-DMG, PEG-DPG, and PEG-DSG), PEG-ceramide, PEG-distearoyl-glycero-phosphoglycerol (PEG-DSPG), PEG-dioleoyl-glycero-phosphoethanolamine (PEG-DOPE), 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, and a PEG-distearoyl-phosphatidylethanolamine (PEG-DSPE) lipid. In some embodiments, the free PEG-lipid comprises a diacylphosphatidylethanolamine, a dialkylphosphatidylethanolamine, a diacylglycerol, a ceramide, a dialkylglycerol, or a dialkylacetamide. In some embodiments, the alkyl chain is myristic acid, palmitic acid, oleic acid, linoleic acid, or stearic acid. In some embodiments, the free PEG-lipid is DMG-PEG. In some embodiments, the free PEG-lipid is DPG-PEG.

In certain embodiments, the blend may contain a free PEG-lipid that can be selected from the group consisting of PEG-distearoylglycerol (PEG-DSG), PEG-diacylglycerol (PEG-DAG, e.g., PEG-DMG, PEG-DPG, and PEG-DSG), PEG-dimyristoyl-glycerol (PEG-DMG), PEG-distearoyl-phosphatidylethanolamine (PEG-DSPE) and PEG-dimyrstoyl-phosphatidylethanolamine (PEG-DMPE). In some embodiments, the free PEG-lipid comprises a diacylphosphatidylcholines comprising Dipalmitoyl (C16) chain or Distearoyl (C18) chain.

The PEG-lipid may be present in the lipid blend in a range of 1-10 mole percent, 1-8 mole percent, 1-7 mole percent, 1-6 mole percent, 1-5 mole percent, 1-4 mole percent, 1-3 mole percent, 2-8 mole percent, 2-7 mole percent, 2-6 mole percent, 2-5 mole percent, 2-4 mole percent, 2-3 mole percent, or about 1 mole percent, about 2 mole percent, about 3 mole percent, about 4 mole percent, or about 5 mole percent. In some embodiments, the free PEG-lipid is present in the lipid blend in a range of 1-4 mole percent, such as about 1-2 mole percent, or about 2-4 mole percent, or about 1.5 mole percent. In some embodiments, the free PEG-lipid is present in the lipid blend in about 0.1-4 mole percent, such as 0.5 to 2.5 mole percent, or about 1 to 2 mole percent. In some embodiments, the PEG-lipid is a free PEG-lipid.

In some embodiments, the PEG-lipid may be present in the lipid blend in the range of 0.01-10 mole percent, 0.01-5 mole percent, 0.01-4 mole percent, 0.01-3 mole percent, 0.01-2 mole percent, 0.01-1 mole percent, 0.1-10 mole percent, 0.1-5 mole percent, 0.1-4 mole percent, 0.1-3 mole percent, 0.1-2 mole percent, 0.1-1 mole percent, 0.5-10 mole percent, 0.5-5 mole percent, 0.5-4 mole percent, 0.5-3 mole percent, 0.5-2 mole percent, 0.5-1 mole percent, 1-2 mole percent, 3-4 mole percent, 4-5 mole percent, 5-6 mole percent, or 1.25-1.75 mole percent. In some embodiments, the PET-lipid may be about 0.5 mole percent, about 1 mole percent, about 1.5 mole percent, about 2 mole percent, about 2.5 mole percent, about 3 mole percent, about 3.5 mole percent, about 4 mole percent, about 4.5 mole percent, about 5 mole percent, or about 5.5 mole percent of the lipid blend. In some embodiments, the PEG-lipid is a free PEG-lipid.

In some embodiments, the lipid anchor length of PEG-lipid is C14 (as in PEG-DMG). In some embodiments, the lipid anchor length of PEG-lipid is C16 (as in DPG). In some embodiments, the lipid anchor length of PEG-lipid is C18 (as in PEG-DSG). In some embodiments, the back

bone or head group of PEG-lipid is diacyl glycerol or phosphoethanolamine. In some embodiments, the PEG-lipid is a free PEG-lipid.

A LNP of the present disclosure may comprise one or more free PEG-lipid that is not conjugated to an immune cell targeting group, and a PEG-lipid that is conjugated to immune cell targeting group. In some embodiments, the free PEG-lipid comprises the same or a different lipid as the lipid in the lipid-immune cell targeting group conjugate.

In some embodiments, the free PEG lipid comprise a PEG having a molecular weight of at least 2000 daltons. In some embodiments, the PEG has a molecular weight of about 3000 to 5000 daltons. In some embodiments, the Fab is an anti-CD3 antibody, and the free PEG lipid in the LNP comprises a PEG having a molecular weight of about 2000 daltons. In some embodiments, the Fab is an anti-CD4 antibody, and the free PEG lipid in the LNP comprises a PEG having a molecular weight of about 3000 to 3500 daltons.

PRODUCTION OF LIPID NANOPARTICLES

In some embodiments, the LNPs are produced by using either rapid mixing by an orbital vortexer or by microfluidic mixing. Orbital vortexer mixing is accomplished by rapid addition of lipids solution in ethanol to the aqueous solution of a nucleic acid of interest followed immediately by vortexing at 2,500 rpm. In some embodiments, the LNPs are produced using a microfluidic mixing step. In some embodiments, microfluidic mixing is achieved mixing the aqueous and organic streams at a controlled flow rates in a microfluidic channel using, e.g., a NanoAssemblr device and microfluidic chips featuring optimized mixing chamber geometry (Precision Nanosystems, Vancouver, BC). In some embodiments, the LNPs are produced using a microfluidic mixing step to rapidly mix the ethanolic lipid solution and aqueous nucleic acid solution, resulting in encapsulation of the nucleic acid in the solid lipid nanoparticles. The nanoparticle suspension is then buffer exchanged into an all aqueous buffer using membrane filtration device of choice for ethanol removal and nanoparticle maturation.

In certain embodiments, the resulting LNP compositions comprise a lipid blend containing, for example, from about 40 mole percent to about 60 mole percent of one or more ionizable cationic lipids described herein, from about 35 mole percent to about 50 mole percent of one or more

sterols, from about 5 mole percent to about 15 mole percent of one or more neutral lipids, and from about 0.5 mole percent to about 5 mole percent of one or more PEG-lipids.

In certain embodiments, the resulting LNP compositions comprise a lipid blend containing, for example, from about 20 mole percent to about 60 mole percent of one or more ionizable cationic lipids described herein, from about 25 mole percent to about 50 mole percent of one or more sterols, from about 15 mole percent to about 60 mole percent of one or more neutral lipids, and from about 0 mole percent to about 5 mole percent of one or more PEG-lipids.

In certain embodiments, the resulting LNP compositions comprise a lipid blend containing, for example, from about 30 mole percent to about 40 mole percent of one or more ionizable cationic lipids described herein, from about 20 mole percent to about 40 mole percent of one or more sterols, from about 30 mole percent to about 50 mole percent of one or more neutral lipids, and from about 0 mole percent to about 5 mole percent of one or more PEG-lipids.

In certain embodiments, the resulting LNP compositions comprise a lipid blend containing, for example, from about 30 mole percent to about 40 mole percent of one or more ionizable cationic lipids described herein, from about 20 mole percent to about 40 mole percent of one or more sterols, from about 30 mole percent to about 50 mole percent of one or more neutral lipids, and from about 0 mole percent to about 5 mole percent of one or more PEG-lipids.

In certain embodiments, the resulting LNP compositions comprise a lipid blend containing, for example, from about 30 mole percent to about 40 mole percent of one or more ionizable cationic lipids described herein, from about 20 mole percent to about 40 mole percent of one or more sterols, from about 30 mole percent to about 50 mole percent of one or more neutral lipids, from about 0 mole percent to about 5 mole percent of one or more PEG-lipids, from about 0.01 to 0.5 PEG-targeting lipid, and from 0 to 0.5 PEG click handle (i.e., azide, DBCO-azide and Tz-TCO).

In certain embodiments, the resulting LNP compositions comprise a lipid blend containing, for example, from about 30 mole percent to about 40 mole percent of one or more ionizable cationic lipids described herein, from about 20 mole percent to about 40 mole percent of one or more sterols, from about 30 mole percent to about 50 mole percent of one or more neutral lipids, from

about 0 mole percent to about 5 mole percent of one or more PEG-lipids, from about 0.03 to 0.05 PEG-targeting lipid, or about 0.1 to 0.2 PEG click handle (i.e., azide, DBCO-azide and Tz-TCO).

In certain embodiments, the resulting LNP compositions comprise a lipid blend containing, for example, from about 30 mole percent to about 40 mole percent of one or more ionizable cationic lipids described herein, from about 20 mole percent to about 40 mole percent of one or more sterols, from about 30 mole percent to about 50 mole percent of one or more neutral lipids, from about 0 mole percent to about 5 mole percent of one or more DSPE PEG FAB, from about 0.03 to 0.05, or from about 0.1 to 0.2 DSPE PEG Azide.

In certain embodiments, the resulting LNP compositions comprise ionizable lipid: structural lipid: sterol lipid: DSG-PEG2000:DSPE-PEG2000-maleimide in a ratio of 58:7:33.5:1.4:0.1 or 58:10:30.5:1.4:0.1.

In certain embodiments, the resulting LNP compositions comprise ionizable lipid: structural lipid: sterol lipid: DPG-PEG2000:DSPE-PEG2000-maleimide in a ratio of 58:7:33.5:1.4:0.1 or 58:10:30.5:1.4:0.1.

In certain embodiments, the resulting LNP compositions comprise ionizable lipid: structural lipid: sterol lipid: PEG-Lipid: DSPE PEG FAB:DSPE PEG Azide at a molar ratio of about 33.0:39.9:25.4:1.54:0.05:0.11 respectively.

In certain embodiments, the resulting LNP compositions comprise ionizable lipid: structural lipid: sterol lipid: PEG-Lipid: DSPE PEG FAB:DSPE PEG Azide at a molar ratio of about 33.5:40.5:25.8:0.0:0.05:0.11 respectively.

In certain embodiments, the lipid containing a targeting moiety is present at less than 2 mol%.

In a certain embodiments, the lipid containing a targeting moiety is present at less than 1.5 mol%.

In certain embodiments, the lipid containing a targeting moiety is present at less than 1.0 mol%.

In certain embodimetns, the lipid containing a targeting moiety is present at less than 0.5 mol%.

PHYSICAL PROPERTIES OF LIPID NANOPARTICLES

The characteristics of an LNP composition may depend on the components, their absolute or relative amounts, contained in a lipid nanoparticle (LNP) composition. Characteristics may also vary depending on the method and conditions of preparation of the LNP composition.

LNP compositions may be characterized by a variety of methods. For example, microscopy (e.g., transmission electron microscopy or scanning electron microscopy) may be used to examine the morphology and size distribution of an LNP composition. Dynamic light scattering or potentiometry (e.g., potentiometric titrations) may be used to measure zeta potentials. Dynamic light scattering may also be utilized to determine particle sizes.

Instruments such as the Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, Worcestershire, UK) may also be used to measure multiple characteristics of an LNP composition, such as particle size, polydispersity index, and zeta potential. RNA encapsulated efficiency is determined by a combination of methods relying on RNA binding dyes (ribogreen, cybergreen to determine dye accessible RNA fraction) and LNP de-formulation followed by HPLC analysis for total RNA content.

In some embodiments, the LNP may have a mean diameter in the range of 1-250 nm, 1-200 nm, 1-150 nm, 1-100 nm, 50-250 nm, 50-200 nm, 50-150 nm, 50-100 nm, 75-250 nm, 75-200 nm, 75-150 nm, 75-100 nm, 100-250 nm, 100-200 nm, 100-150 nm. In certain embodiments, the LNP compositions may have a mean diameter of about 1nm, about 10 nm, about 20 nm, about 30 nm, about 40 nm, about 50 nm, about 60 nm, about 70 nm, about 80 nm, about 90 nm, about 100 nm, about 110 nm, about 120 nm, about 130 nm, about 140 nm, about 150 nm, about 160 nm, about 170 nm, about 180 nm, about 190 nm, or about 200 nm. In some embodiments, the LNP has a mean diameter of about 100 nm. In some embodiments, the LNP has a mean diameter in the range of 50-200 nm.

In some embodiments, LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, show average diameter change after a freeze-thaw of less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or 40%. In some embodiments,

LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, show average diameter change after a freeze-thaw of less than 30%. In some embodiments, the freeze-thaw and diameter measurements are conducted with 10% sucrose in MES pH 6.5 buffer.

In some embodiments, LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, show average diameter change upon targeting antibody insertion of less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or 40%. In some embodiments, LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, show average diameter change upon targeting antibody insertion of less than 15%. In some embodiments, the diameter change upon targeting antibody insertion is measured in pH 6.5 MES using a 37°C incubation for 4 hours.

In some embodiments, LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, have average LNP diameter of less than 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 nm. In some embodiments, LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, have average LNP diameter of less than 100 nm.

Alternatively or in addition, the LNP compositions may have a polydispersity index in a range from 0.05-1, 0.05-0.75, 0.05-0.5, 0.05-0.4, 0.05-0.3, 0.05-0.2, 0.08-1, 0.08-0.75, 0.08-0.5, 0.08-0.4, 0.08-0.3, 0.08-0.2, 0.1-1, 0.1-0.75, 0.1-0.5, 0.1-0.4, 0.1-0.3, 0.1-0.2. In certain embodiments, the polydispersity index is in the range of 0.1-0.25, 0.1-0.2, 0.1-0.19, 0.1-0.18, 0.1-0.17, 0.1-0.16, or 0.1-0.15. In some embodiments, the LNP has a polydispersity index in a range from 0.05 to 1.

In some embodiments, the LNP compositions or LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, have polydispersity of less than 0.4, 0.3, 0.25, 0.2, 0.15, 0.1, or 0.05. In some embodiments, LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, have polydispersity of less than 0.25.

Alternatively or in addition, the LNP compositions may have a zeta potential of about -30 mV to about +30 mV. In certain embodiments, the LNP composition has a zeta potential of about -10 mV to about +20 mV. The zeta potential may vary as a function of pH. As a result, in certain embodiments, the LNP compositions may have a zeta potential of about 0 mV to about + 30 mV or about +10 mV to + 30 mV or about + 20 mV to about + 30 mV at pH 5.5 or pH 5, and/or a zeta potential of about -30 mV to about + 5 mV or about – 20 mV to about + 15 mV at pH 7.4. In some embodiments, the LNP has a zeta potential of from about +5 mV to about +50 mV at pH5, such as about +10 mV to about + 30 mV at pH 5. In some embodiments, the LNP has a zeta potential of from about –10 mV to about +10 mV at pH 7.4.

In some embodiments, the LNP compositions or LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, have Zeta Potential at pH 7.4 greater than -10, -9, -8, -7, -6, -5.5, -5, -4.5, -4, -3.5, -3, -2.5, -2, -1.5, -1, or -0.5 mV. In some embodiments, the LNP compositions LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, have Zeta Potential at pH 7.4 greater than -10 mV. In some embodiments, the LNP compositions LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, have Zeta Potential at pH 7.4 greater than -1 mV. In some embodiments, the LNP compositions LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, have Zeta Potential at pH 5.5 greater than -1, 0, 1, 2, 3, 4, 4.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, or 25 mV. In some embodiments, the LNP compositions LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, have Zeta Potential at pH 5.5 greater than 5 mV. In some embodiments, the LNP compositions LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, have Zeta Potential at pH 5.5 greater than 15 mV.

V. PAYLOADS

The LNP compositions may comprise an agent, for example, a nucleic acid molecule for delivery to a cell (e.g., an immune cell) or tissue, for example, a cell (e.g., an immune cell) or tissue in a subject. In certain embodiments, the number of the nucleotides in the nucleic acid is from about 400 to about 6000.

The LNP compositions of the present invention may include a nucleic acid, for example, a DNA or RNA, such as an mRNA, tRNA, microRNA, siRNA, gRNA (guide RNA), circRNA (circular RNA), ribozymes, decoy RNA or dicer substrate siRNA. In some embodiments, the mRNA encodes a receptor, a growth factor, a hormone, a cytokine, an antibody, an antigen, an enzyme, or a vaccine. In some embodiments, the mRNA encodes a polypeptide capable of regulating immune response in the immune cell. In some embodiments, the mRNA encodes a polypeptide capable of reprogramming the immune cell. In some embodiments, the mRNA encodes a synthetic T cell receptor (synTCR) or a Chimeric Antigen Receptor (CAR). In some embodiments, the CAR is TTR-023 anti-CD20 (Leu-16). In some embodiments, the CAR comprises the amino acid sequence of SEQ ID NO: 24. In some embodiments, the mRNA encoding the CAR comprises the polynucleotide sequence of 25. TTR-023 anti-CD20 (Leu-16) CAR sequence (including leader) (SEQ ID NO: 24):

METDTLLLWVLLLWVPGSTGDYKAKEVQLQQSGAELVKPGASVKMSCKASGYTFT
SYNMHWVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSS
LTSEDSADYYCARSNYYGSSYWFFDVWGAGTTVTVSSGGGSGGGSGGGGSSDIVLT
QSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPARFS
GSGSGTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGGGGSAAAIEVMY
PPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIF
WVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSAEPPAYQQ
GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYS
EIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 24)

Corresponding nucleic acid sequence (SEQ ID NO: 25):
atggagaccgacaccctgttgctttgggtactgttacttlgggtgccccggatctaccggtgattacaaggccaaggaggtgcagctgca
gcagagcggagccgagctggtgaagccaggcgcttccgtgaagatgtcttgtaaggcctccggctacacattcaccagctacaatat
gcactgggtaaagcagactccggggcagggcctggagtggataggtgccatctaccctggcaacggcgacaccagctacaaccag
aagtttaaggggaaggctactctaacagcggacaagtcgtcctctaccgcctacatgcaactcagctccctgacgagcgaggactcc
gcggactactactgtgcccgctccaactactacggctctagctattggttcttcgacgtgtggggcgctggaacgaccgtgaccgtgtct
tccggtggaggttccgggggcggaagcggcggtggcggcagttcggacatcgtgctgacccagagccctgccatcctgtccgcttc
cccgggggagaaagttacgatgacctgccgagcgagctccagtgtcaactacatggattggtaccagaagaagccccggcagcagtc
ccaagccgtggatttacgctactagcaacctggcgtccggtgtcccggctcgcttctcaggttctggctcgggtactagttattcattaac

catttctcgcgtggaggctgaggacgctgccacctactactgccaacagtggtctttcaaccctcccactttcggaggcggcaccaagc
tcgagatcaagggcgggggtggctccgcagcagccattgaggtgatgtatcctcctccctatttggacaacgagaagtcaaatggcac
catcatccacgttaagggcaagcacctgtgcccatctcccctgttcccaggcccctctaagcccttctgggtcctggtggtggtcggcg
gcgtcctggcatgttactctctgctggtgaccgtcgcgttcatcatcttttgggtccggtccaagcgcagccgcctgctccactccgacta
catgaatatgactcctcgtaggcccggtccaacccgcaagcactaccagccgtacgcgccgcccagagactttgctgcttaccgatcc
agagtgaaattttctaggtcggccgaacctcccgcatatcagcagggccagaaccagctgtacaacgaactcaacttgggacggcgc
gaggaatacgatgtgctggataaacgccgtggccgcgatcccgagatgggcgggaagccacgtcgcaaaaaccctcaggagggc
ctttacaacgagttgcagaaggacaaaatggcggaggcctactccgagatcggaatgaaggggagcgccggcgcggcaaaggg
catgacggcctctaccagggcctgtccacagccacgaaagacacctatgacgccctgcatatgcaggccctgccccccgcgctgataa
tga (SEQ ID NO: 25)

It is contemplated that nucleic acids can contain naturally occurring components, such as, naturally occurring bases, sugars or linkage groups (e.g., phosphodiester linkage groups) or may contain non-naturally occurring components or modifications, (e.g., thioester linkage groups). For example, the nucleic acid can be synthesized to contain base, sugar, linker modifications known to those skilled in the art. Furthermore, the nucleic acids can be linear or circular, or have any desired configuration. The LNP compositions can include multiple nucleic acid molecules, for example, multiple RNA molecules, which can be the same or different.

In certain embodiments, the payload is an mRNA. In certain embodiments, a particular LNP composition may contain a number of mRNA molecules that can be the same or different. In certain embodiments, one or more LNP compositions including one or more different mRNAs may be combined, and/or simultaneously contacted, with a cell. It is contemplated that an mRNA may include one or more of a stem loop, a chain terminating nucleoside, a polyA sequence, a polyadenylation signal, and/or a 5' cap structure. The mRNA may encode a receptor, such as a chimeric antigen receptor (CAR), for use in for example, an immune disorder, inflammatory disorder or cancer. In addition, the mRNA may encode an antigen for use in a therapeutic or prophylactic vaccine, for example, for treating or preventing an infection by a pathogen, for example, a microbial or viral pathogen, or for reducing or ameliorating the side effects caused directly or indirectly by such an infection.

In certain embodiment, the CAR is selected from:

Signal peptide	α-CD19 svFv (source)	Hinge + TM	Co-stimulatory domain	Signalling domain
CD8α	Murine	CD28	CD28	CD3zeta
CD8α	Human	CD8α	4-1BB	CD3zeta
CD8α	Human	CD28	CD28	CD3zeta
TCRβ	Blinatumomab	CD8α	4-1BB	CD3zeta
TCRβ	Blinatumomab	CD28	4-1BB	CD3zeta
TCRβ	Blinatumomab	$CH2CH3^{+}$ CD8α	4-1BB	CD3zeta
TCRβ	Xmab5774	CD8α	4-1BB	CD3zeta

In certain embodiments, the CAR is selected from:

Leader Seq	scfv	hinge	TM	co-stim ICD	stim ICD
hCD8a	FMC63	hCD8a	hCD8a	h41BB	hCd3z
hCSF2RA	FMC63	hIgG4	hCd28	h41BB	hCd3z
hCD8a	human CD19 scfv	hCD8a	hCD8a	hCD28	hCd3z
hCD8a	FMC63	hCD8a	hCD8a	hCD28	hCd3z
hCSF2RA	FMC63	hIgG4	hCd28	hCD28	hCd3z
hCD8a	human CD19 scfv	hCD8a	hCD8a	h41BB	hCd3z
hCD8a	human CD19 scfv	hCD8a	hCd28	h41BB	hCd3z
hCD8a	humanCD19 scfv	hIgG4	hCd28	h41BB	hCd3z
hCSF2RA	human CD19 scfv	hIgG4	hCd28	hCD28	hCd3z

In certain embodiments, the LNP composition may include one or more other components including, but not limited to, one or more pharmaceutically acceptable excipients, small

hydrophobic molecules, therapeutic agents, carbohydrates, polymers, permeability enhancing molecules, and surface altering agents.

In some embodiments, the wt/wt ratio of the lipid component to the payload (e.g., mRNA) in the resulting LNP composition is from about 1:1 to about 50:1. In certain embodiments, the wt/wt ratio of the lipid component to the payload (e.g., mRNA) in the resulting composition is from about 5:1 to about 50:1. In certain embodiments, the wt/wt ratio is from about 5:1 to about 40:1. In certain embodiments, the wt/wt ratio is from about 10:1 to about 40:1. In certain embodiments, the wt/wt ratio is from about 15:1 to about 25:1.

In certain embodiments, the encapsulation efficiency of the payload (e.g., mRNA) in the lipid nanoparticles is at least 50%. In certain embodiments, the encapsulation efficiency is at least 80%, at least 90% or, or greater than 90%.

In some embodiments, LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, exhibit encapsulation efficiency of greater than 50, 55, 60, 65, 70, 75, 80, 82.5, 85, 87.5, 90, 92.5, 95, 97.5, or 99%. In some embodiments, LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, exhibit encapsulation efficiency of greater than 87.5%. In some embodiments, LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, exhibit dye accessible RNA of less than 50, 45, 40, 35, 30, 25, 20, 17.5, 15, 12.5, 10, 7.5, 5, 2.5, or 1%. In some embodiments, LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, exhibit dye accessible RNA of less than 12.5%.

In some embodiments, LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, exhibit total mRNA recovery of greater than 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95%. In some embodiments, LNPs comprising an ionizable cationic lipid described herein, prepared and characterized using methods described herein, exhibit total mRNA recovery of greater than 80%.

RNA PAYLOAD

In certain embodiments, the RNA payload is an mRNA, tRNA, microRNA, or siRNA payload.

In certain embodiments, the lipid nanoparticle compositions are optimized for the delivery of RNA, e.g., mRNA, to a target cell for translation within the cell. An mRNA may be a naturally or non-naturally occurring mRNA. An mRNA may include one or more modified nucleobases, nucleosides, or nucleotides.

The nucleobases may be selected from the non-limiting group consisting of adenine, guanine, uracil, cytosine, 7-methylguanine, 5-methylcytosine, 5-hydroxymethylcytosine, thymine, pseudouracil, dihydrouracil, N1-methylpseudouracil, hypoxanthine, and xanthine. In some embodiments, nucleobase is N1-methylpseudouracil.

A nucleoside of an mRNA is a compound including a sugar molecule (e.g., a 5-carbon or 6-carbon sugar, such as pentose, ribose, arabinose, xylose, glucose, galactose, or a deoxy derivative thereof) in combination with a nucleobase. A nucleoside may be a canonical nucleoside (e.g., adenosine, guanosine, cytidine, uridine, 5-methyluridine, deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine, and thymidine) or an analog thereof and may include one or more substitutions or modifications.

A nucleotide of an mRNA is a compound containing a nucleoside and a phosphate group or alternative group (e.g., boranophosphate, thiophosphate, selenophosphate, phosphonate, alkyl group, amidate, and glycerol). A nucleotide may be a canonical nucleotide (e.g., adenosine, guanosine, cytidine, uridine, 5-methyluridine, deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine, and thymidine monophosphates) or an analog thereof and may include one or more substitutions or modifications including but not limited to alkyl, aryl, halo, oxo, hydroxyl, alkyloxy, and/or thio substitutions; one or more fused or open rings; oxidation; and/or reduction of the nucleobase, sugar, and/or phosphate or alternative component. A nucleotide may include one or more phosphate or alternative groups. For example, a nucleotide may include a nucleoside and a triphosphate group. A "nucleoside triphosphate" (e.g., guanosine triphosphate, adenosine triphosphate, cytidine triphosphate, and uridine triphosphate) may refer to the canonical nucleoside triphosphate or an analog or derivative thereof and may include one or more substitutions or modifications as described herein.

An mRNA may include a 5' untranslated region, a 3' untranslated region, and/or a coding or translating sequence. An mRNA may include any number of base pairs, including tens,

hundreds, or thousands of base pairs. Any number (e.g., all, some, or none) of nucleobases, nucleosides, or nucleotides may be an analog of a canonical species, substituted, modified, or otherwise non-naturally occurring. In certain embodiments, all of a particular nucleobase type may be modified. For example, all cytosine in an mRNA may be 5-methylcytosine. In certain embodiments, one or more or all uridine bases may be N1-methylpseudouridines.

In certain embodiments, an mRNA may include a 5' cap structure, a chain terminating nucleotide, a stem loop, a polyA sequence, and/or a polyadenylation signal.

A cap structure or cap species is a compound including two nucleoside moieties joined by a linker and may be selected from a naturally occurring cap, a non-naturally occurring cap or a cap analog. A cap species may include one or more modified nucleosides and/or linker moieties. For example, a natural mRNA cap may include a guanine nucleotide and a guanine (G) nucleotide methylated at the 7 position joined by a triphosphate linkage at their 5' positions, e.g., m7G(5')ppp(5')G, commonly written as m7GpppG. A cap species may also be an anti-reverse cap analog. A non-limiting list of possible cap species includes m7GpppG, m7Gpppm7G, m73'dGpppG, m7Gpppm7G, m73'dGpppG, and m2702'GppppG.

Alternatively or in addition, an mRNA may include a chain terminating nucleoside. For example, a chain terminating nucleoside may include those nucleosides deoxygenated at the 2' and/or 3' positions of their sugar group. Such species may include 3'-deoxyadenosine (cordycepin), 3'-deoxyuridine, 3'-deoxycytosine, 3'-deoxyguanosine, 3'-deoxythymine, and 2',3'-dideoxynucleosides, such as 2',3'-dideoxyadenosine, 2',3'-dideoxyuridine, 2',3'-dideoxycytosine, 2',3'-dideoxyguanosine, and 2',3'-dideoxythymine.

Alternatively or in addition, an mRNA may include a stem loop, such as a histone stem loop. A stem loop may include 1, 2, 3, 4, 5, 6, 7, 8, or more nucleotide base pairs. For example, a stem loop may include 4, 5, 6, 7, or 8 nucleotide base pairs. A stem loop may be located in any region of an mRNA. For example, a stem loop may be located in, before, or after an untranslated region (a 5' untranslated region or a 3' untranslated region), a coding region, or a polyA sequence or tail.

Alternatively or in addition, an mRNA may include a polyA sequence and/or polyadenylation signal. A polyA sequence may be comprised entirely or mostly of adenine nucleotides or analogs

or derivatives thereof. A polyA sequence may be a tail located adjacent to a 3' untranslated region of an mRNA.

An mRNA may encode any polypeptide of interest, including any naturally or non-naturally occurring or otherwise modified polypeptide. A polypeptide encoded by an mRNA may be of any size and may have any secondary structure or activity. In some embodiments, a polypeptide encoded by an mRNA may have a therapeutic effect when expressed in a cell. In some embodiments, the mRNA may encode an antibody, enzyme, growth factor, hormone, cytokine, viral protein (e.g., a viral capsid protein), antigen, vaccine, or receptor. In some embodiments, the mRNA may encode an engineered receptor such as a CAR or an antigen for use in a therapeutic vaccine (e.g., a cancer vaccine) or a prophylactic vaccine (e.g., a vaccine for minimizing the risk or severity of an infection by a microbial or viral pathogen). In some embodiments, the mRNA encodes a polypeptide capable of regulating immune response in the immune cell. In some embodiments, the mRNA encodes a polypeptide capable of reprogramming the immune cell. In some embodiments, the mRNA encodes a synthetic T cell receptor (synTCR) or a Chimeric Antigen Receptor (CAR).

A lipid composition may be designed for one or more specific applications or targets. For example, an LNP composition may be designed to deliver mRNA to a particular cell, tissue, organ, or system or group thereof in a mammal's body, such as the renal system. Physiochemical properties of LNP compositions may be altered in order to increase selectivity for particular target site within a subject. For instance, particle sizes may be adjusted based on the fenestration sizes of different organs. The mRNA included in an LNP composition may also depend on the desired delivery target or targets. For example, an mRNA may be selected for a particular indication, condition, disease, or disorder and/or for delivery to a particular cell, tissue, organ, or system or group thereof (e.g., localized or specific delivery).

The amount of mRNA in a lipid composition may depend on the size, sequence, and/or other characteristics of the mRNA. The amount of mRNA in an LNP may also depend on the size, composition, desired target, and/or other characteristics of the LNP composition. The relative amounts of mRNA and other elements (e.g., lipids) may also vary. The amount of mRNA in an

LNP composition may, for example, be measured using absorption spectroscopy (e.g., ultraviolet-visible spectroscopy).

In some embodiments, the one or more mRNAs, lipids, and polymers and amounts thereof may be selected to provide a specific N:P ratio (the ratio of positively-chargeable lipid or polymer amine (N = nitrogen) groups to negatively-charged nucleic acid phosphate (P) groups). The N:P ratio of the composition refers to the molar ratio of nitrogen atoms in one or more lipids to the number of phosphate groups in an mRNA. In general, a lower N:P ratio is preferred. A N:P ratio may be dependent on a specific lipid and its pKa. In certain embodiments, the mRNA and LNP composition, and/or their relative amounts may be selected to provide an N:P ratio from about 1:1 to about 30:1, or from about 1:1 to about 20:1. In certain embodiments, the N:P ratio can be, for example, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, or 8:1. In certain embodiments, the N:P ratio may be from about 2:1 to about 5:1. In certain embodiments, the N:P ratio may be about 4:1. In other embodiments, the N:P ratio is from about 4:1 to about 8:1. For example, the N:P ratio may be about 4:1, about 4.5:1, about 4.6:1, about 4.7:1, about 4.8:1, about 4.9:1, about 5.0:1, about 5.1:1, about 5.2:1, about 5.3:1, about 5.4:1, about 5.5:1, about 5.6:1, about 5.7:1, about 6.0:1, about 6.5:1, or about 7.0:1.

The amount of mRNA in a nanoparticle composition may depend on the size, sequence, and/or other characteristics of the mRNA. The amount of mRNA in a nanoparticle composition may also depend on the size, composition, desired target, and/or other characteristics of the nanoparticle composition. The relative amounts of mRNA and other elements (e.g., lipids) may also vary. In some embodiments, the wt/wt ratio of the lipid component to an mRNA in a nanoparticle composition may be from about 5:1 to about 50:1, such as 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, and 50:1. For example, the wt/wt ratio of the lipid component to an mRNA may be from about 10:1 to about 40:1. The amount of mRNA in a nanoparticle composition may, for example, be measured using absorption spectroscopy (e.g., ultraviolet-visible spectroscopy).

The efficiency of encapsulation of an mRNA describes the amount of mRNA that is encapsulated or otherwise associated with a lipid composition after preparation, relative to the initial amount provided. The encapsulation efficiency is desirably high (e.g., close to 100%). The

encapsulation efficiency may be measured, for example, by comparing the amount of mRNA in a solution containing the lipid composition before and after breaking up the LNP composition with one or more organic solvents or detergents. Fluorescence may be used to measure the amount of free mRNA in a solution. For the LNP compositions of the invention, the encapsulation efficiency of an mRNA may be at least 50%, for example 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. In certain embodiments, the encapsulation efficiency may be at least 80%.

VI. FORMULATION AND MODE OF DELIVERY

LNP compositions of the invention may be formulated in whole or in part as a pharmaceutical composition. The pharmaceutical compositions may further include one or more pharmaceutically acceptable excipients or accessory ingredients such as those described herein. General guidelines for the formulation and manufacture of pharmaceutical compositions and agents are available, for example, in Remington's (2006) supra. Conventional excipients and accessory ingredients may be used in any pharmaceutical composition of the invention, except insofar as any conventional excipient or accessory ingredient may be incompatible with one or more components of an LNP composition of the invention. An excipient or accessory ingredient may be incompatible with a component of an LNP composition if its combination with the component may result in any undesirable biological effect or otherwise deleterious effect.

In some embodiments, one or more excipients or accessory ingredients may make up greater than 50% of the total mass or volume of a pharmaceutical composition including an LNP composition of the invention. For example, the one or more excipients or accessory ingredients may make up 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of a pharmaceutical composition. In certain embodiments, the excipient is approved for use in humans and for veterinary use, for example, by United States Food and Drug Administration. In certain embodiments, the excipient is pharmaceutical grade. In certain embodiments, an excipient meets the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

Relative amounts of the one or more lipids or LNPs, one or more pharmaceutically acceptable excipients, and/or any additional ingredients in a pharmaceutical composition will vary,

depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered.

Lipid compositions and/or pharmaceutical compositions including one or more LNP compositions may be administered to any subject, including a human patient that may benefit from a therapeutic effect provided by the delivery of a nucleic acid, e.g., an RNA (e.g., mRNA, tRNA or siRNA) to one or more particular cells, tissues, organs, or systems or groups thereof, such as the renal system. Although the descriptions provided herein of LNP compositions and pharmaceutical compositions including LNP compositions are principally directed to compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other mammal. Modification of compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is understood.

A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient (e.g., the payload).

Pharmaceutical compositions of the invention may be prepared in a variety of forms suitable for a variety of routes and methods of administration. For example, pharmaceutical compositions of the invention may be prepared in liquid dosage forms (e.g., emulsions, microemulsions, nanoemulsions, solutions, suspensions, syrups, and elixirs), injectable forms, solid dosage forms (e.g., capsules, tablets, pills, powders, and granules), dosage forms for topical and/or transdermal administration (e.g., ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants, and patches), suspensions, powders, and other forms.

Liquid dosage forms for oral and parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, nanoemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils

(in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and/or perfuming agents.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing agents, wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables.

Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

Other Components

In addition, it is contemplated that the pharmaceutical compositions may include one or more components in addition to those described hereinabove.

The pharmaceutical compositions may also include one or more permeability enhancer molecules, carbohydrates, polymers, therapeutic agents, surface altering agents, or other components. A permeability enhancer molecule may be a molecule described, for example, in U.S. patent application publication No.2005/0222064. Carbohydrates may include simple sugars (e.g., glucose) and polysaccharides (e.g., glycogen and derivatives and analogs thereof).

The pharmaceutical compositions may also contain a surface altering agent, including for example, anionic proteins (e.g., bovine serum albumin), surfactants (e.g., cationic surfactants such as dimethyldioctadecyl-ammonium bromide), sugars or sugar derivatives (e.g., cyclodextrin), polymers (e.g., heparin, polyethylene glycol, and poloxamer), mucolytic agents

(e.g., acetylcysteine, mugwort, bromelain, papain, clerodendrum, bromhexine, carbocisteine, eprazinone, mesna, ambroxol, sobrerol, domiodol, letosteine, stepronin, tiopronin, gelsolin, thymosin β4, dornase alfa, neltenexine, and erdosteine), and DNases (e.g., rhDNase). A surface altering agent may be disposed within and/or upon the surface of a composition described herein.

In addition to these components, a pharmaceutical composition containing an LNP composition of the invention may include any substance useful in pharmaceutical compositions. For example, the pharmaceutical composition may include one or more pharmaceutically acceptable excipients or accessory ingredients such as, but not limited to, one or more solvents, dispersion media, diluents, dispersion aids, suspension aids, granulating aids, disintegrants, fillers, glidants, liquid vehicles, binders, surface active agents, isotonic agents, thickening or emulsifying agents, buffering agents, lubricating agents, oils, preservatives, and other species. Excipients such as waxes, butters, coloring agents, coating agents, flavorings, and perfuming agents may also be included. Pharmaceutically acceptable excipients are well known in the art (see, e.g., Remington's (2006) supra).

Dispersing agents may be selected from the non-limiting list consisting of potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (VEEGUM®), sodium lauryl sulfate, quaternary ammonium compounds, and/or combinations thereof.

Surface active agents and/or emulsifiers may include, but are not limited to, natural emulsifiers (e.g., acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (e.g., bentonite [aluminum silicate] and VEEGUM® [magnesium aluminum silicate]), long chain amino acid derivatives, high molecular weight alcohols (e.g., stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene

glycol monostearate, polyvinyl alcohol), carbomers (e.g., carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (e.g., carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (e.g., polyoxyethylene sorbitan monolaurate [TWEEN®20], polyoxyethylene sorbitan [TWEEN® 60], polyoxyethylene sorbitan monooleate [TWEEN®80], sorbitan monopalmitate [SPAN®40], sorbitan monostearate [SPAN®60], sorbitan tristearate [SPAN®65], glyceryl monooleate, sorbitan monooleate [SPAN®80]), polyoxyethylene esters (e.g., polyoxyethylene monostearate [MYRJ® 45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and SOLUTOL®), sucrose fatty acid esters, polyethylene glycol fatty acid esters (e.g., CREMOPHOR®), polyoxyethylene ethers, (e.g., polyoxyethylene lauryl ether [BRIJ® 30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, PLURONIC®F 68, POLOXAMER® 188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, and/or combinations thereof.

Examples of preservatives may include, but are not limited to, antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and/or other preservatives. Examples of antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and/or sodium sulfite. Examples of chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium edetate, tartaric acid, and/or trisodium edetate. Examples of antimicrobial preservatives include, but are not limited to, benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and/or thimerosal. Examples of antifungal preservatives include, but are not limited to, butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and/or sorbic acid. Examples of alcohol

preservatives include, but are not limited to, ethanol, polyethylene glycol, benzyl alcohol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and/or phenylethyl alcohol. Examples of acidic preservatives include, but are not limited to, vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroascorbic acid, ascorbic acid, sorbic acid, and/or phytic acid. Other preservatives include, but are not limited to, tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite.

Examples of buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, d-gluconic acid, calcium glycerophosphate, calcium lactate, calcium lactobionate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, amino-sulfonate buffers (e.g., HEPES), magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, and/or combinations thereof.

In certain embodiments, the lipid nanoparticle compositions and formulations thereof are adapted for administration intravenously, intramuscularly, intradermally, subcutaneously, intra-arterially, intra-tumor, or by inhalation. In certain embodiments, a dose of about 0.001 mg/kg to about 10 mg/kg is administered to a subject. Compositions in accordance with the present disclosure may be formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of a composition of the present disclosure will be decided by an attending physician within the scope of sound medical judgment.

The specific therapeutically effective, prophylactically effective, or otherwise appropriate dose level (e.g., for imaging) for any particular patient will depend upon a variety of factors including the severity and identify of a disorder being treated, if any; the one or more mRNAs employed; the specific composition employed; the age, body weight, general health, sex, and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific pharmaceutical composition employed; the duration of the treatment; drugs used in combination or coincidental with the specific pharmaceutical composition employed; and like factors well known in the medical arts.

VII. METHODS

The present disclosure provides methods of delivering a payload to a target cell or tissue, for example, a target cell or tissue in a subject, and LNPs or pharmaceutical compositions containing the LNPs for use in such methods. Any disclosure herein of a method of, e.g., treating a disease or disorder or, e.g., delivering a nucleic acid to a cell or, e.g., producing a polypeptide of interest in a cell should be interpreted also as a disclosure of an LNP or pharmaceutical composition comprising said LNP for use in such methods.

In another aspect, provided herein is a method of delivering a nucleic acid to an immune cell (e.g., a T-cell), the method comprising exposing the immune cell to an LNP described herein containing the nucleic acid under conditions that permit the nucleic acid to enter the immune cell.

In another aspect, provided herein is a method of delivering a nucleic acid to a cell, e.g., an immune cell (e.g., a T-cell) in a subject in need thereof, the method comprising administering to the subject a composition comprising an LNP described herein containing a nucleic acid thereby to deliver the nucleic acid to the immune cell.

In another aspect, provided herein is a method of targeting the delivering of a nucleic acid (e.g., mRNA) to a cell, e.g., an immune cell (e.g., a T-cell) in a subject, the method comprising administering to the subject an LNP described herein containing the nucleic acid so as to facilitate targeted delivery of the nucleic acid to the immune cell.

In certain embodiments, the invention provides a method of producing a polypeptide of interest (e.g., a protein of interest) in a mammalian cell, and LNPs or pharmaceutical compositions containing the LNPs for use in such methods. Methods of producing polypeptides in such a cell involve contacting a cell with an LNP composition comprising an RNA of interest (e.g., an mRNA encoding the polypeptide of interest (e.g., a protein of interest). Upon contacting the cell with the LNP composition, the mRNA may be taken up and translated in the cell to produce the polypeptide of interest.

In general, the step of contacting a mammalian cell with an LNP composition including an mRNA encoding a polypeptide of interest may be performed *in vivo*, *ex vivo*, or *in vitro*. The amount of an LNP composition contacted with a cell, and/or the amount of mRNA therein, may depend on the type of cell or tissue being contacted, the means of administration, the physiochemical characteristics of the LNP composition and the mRNA (e.g., size, charge, and chemical composition) therein, and other factors. In general, an effective amount of the LNP composition will allow for efficient polypeptide production in the cell. Metrics for efficiency may include polypeptide translation (indicated by polypeptide expression), level of mRNA degradation, and immune response indicators.

The step of contacting an LNP composition including an mRNA with a cell may involve or cause transfection where the LNP composition may fuse with the membrane of cell to permit the delivery of the mRNA into the cell. Upon introduction into the cytoplasm of the cell, the mRNA is then translated into a protein or peptide via the protein synthesis machinery within the cytoplasm of the cell.

In certain embodiments, the LNP compositions described herein may be used to deliver therapeutic or prophylactic agents to a subject. For example, an mRNA included in an LNP composition may encode a polypeptide and produce the therapeutic or prophylactic polypeptide upon contacting and/or entry (e.g., transfection) into a cell. In certain embodiments, an mRNA included in an LNP composition of the invention may encode a polypeptide that may improve or increase the immunity of a subject.

In certain embodiments, contacting a cell with an LNP composition including an mRNA may reduce the innate immune response of a cell to an exogenous nucleic acid. A cell may be

contacted with a first LNP composition including a first amount of a first exogenous mRNA including a translatable region and the level of the innate immune response of the cell to the first exogenous mRNA may be determined. Subsequently, the cell may be contacted with a second composition including a second amount of the first exogenous mRNA, the second amount being a lesser amount of the first exogenous mRNA compared to the first amount. Alternatively, the second composition may include a first amount of a second exogenous mRNA that is different from the first exogenous mRNA. The steps of contacting the cell with the first and second compositions may be repeated one or more times.

Additionally, efficiency of polypeptide production in the cell may be optionally determined, and the cell may be re-contacted with the first and/or second composition repeatedly until a target protein production efficiency is achieved.

The present disclosure provides methods of delivering a nucleic acid (e.g., an mRNA) to a mammalian cell or tissue, for example, a mammalian cell or tissue in a subject. Delivery of an mRNA to such a cell or tissue involves administering an LNP composition including the mRNA to a subject, for example, by injection, e.g., via intramuscular injection or intravascular delivery into the subject. After administration, the LNP can target and/or contact a cell, for example, an immune cell, such as a T-cell. Upon contacting the cell with the LNP composition, a translatable mRNA may be translated in the cell to produce a polypeptide of interest.

In certain embodiments, an LNP composition of the invention may target a particular type or class of cells. This targeting may be facilitated using the lipids described herein to form LNPs, which may also include a targeting group for targeting cells of interest. In certain embodiments, specific delivery may result in a greater than 2 fold, 5 fold, 10 fold, 15 fold, or 20 fold increase in the amount of mRNA to the targeted destination (e.g., cells that express or express at high levels the receptor of interest which binds to the immune cell targeting group of the LNPs) as compared to another destinations (e.g., cells that either do not express or only express at low levels the receptor of interest).

LNP compositions of the invention may be useful for treating a disease, disorder, or condition characterized by missing or aberrant protein or polypeptide activity. Upon delivery of an mRNA encoding the missing or aberrant polypeptide to a cell, translation of the mRNA may produce the

polypeptide, thereby reducing or eliminating an issue caused by the absence of or aberrant activity caused by the polypeptide. Because translation may occur rapidly, the methods and compositions of the invention may be useful in the treatment of acute diseases, disorders, or conditions such as sepsis, stroke, and myocardial infarction. An mRNA included in an LNP composition of the invention may also be capable of altering the rate of transcription of a given species, thereby affecting gene expression.

Diseases, disorders, and/or conditions characterized by dysfunctional or aberrant protein or polypeptide activity for which a composition of the invention may be administered include, but are not limited to, cancer and proliferative diseases, genetic diseases (e.g., cystic fibrosis), autoimmune diseases, diabetes, neurodegenerative diseases, cardio- and reno-vascular diseases, and metabolic diseases. Multiple diseases, disorders, and/or conditions may be characterized by missing (or substantially diminished such that proper protein function does not occur) protein activity. Such proteins may not be present, or they may be essentially non-functional. A specific example of a dysfunctional protein is the missense mutation variants of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which produce a dysfunctional protein variant of CFTR protein, which causes cystic fibrosis. The present disclosure provides a method for treating such diseases, disorders, and/or conditions in a subject by administering an LNP composition including an mRNA and a lipid component including KL10, a phospholipid (optionally unsaturated), a PEG lipid, and a structural lipid, wherein the m RNA encodes a polypeptide that antagonizes or otherwise overcomes an aberrant protein activity present in the cell of the subject.

The therapeutic and/or prophylactic compositions described herein may be administered to a subject using any reasonable amount and any route of administration effective for preventing, treating, diagnosing, or imaging a disease, disorder, and/or condition and/or any other purpose. The specific amount administered to a given subject may vary depending on the species, age, and general condition of the subject, the purpose of the administration, the particular composition, the mode of administration, and the like. Compositions in accordance with the present disclosure may be formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of a composition of the present disclosure will be decided by an attending physician within the scope of sound medical judgment.

A LNP composition including one or more mRNAs may be administered by a variety of routes, for example, orally, intravenously, intramuscularly, intra-arterially, intramedullary, intrathecally, subcutaneously, intraventricularly, trans- or intra-dermally, intradermally, rectally, intravaginally, intraperitoneally, topically, mucosally, nasally, or intratumorally. In certain embodiments, an LNP composition may be administered intravenously, intramuscularly, intradermally, intra-arterially, intratumorally, or subcutaneously. However, the present disclosure encompasses the delivery of LNP compositions of the invention by any appropriate route taking into consideration likely advances in the sciences of drug delivery. In general, the most appropriate route of administration will depend upon a variety of factors including the nature of the LNP composition including one or more mRNAs (e.g., its stability in various bodily environments such as the bloodstream and gastrointestinal tract), the condition of the patient (e.g., whether the patient is able to tolerate particular routes of administration), etc.

LNP compositions including one or more mRNAs may be used in combination with one or more other therapeutic, prophylactic, diagnostic, or imaging agents. By "in combination with," it is not intended to imply that the agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of the present disclosure. For example, one or more LNP compositions including one or more different m RNAs may be administered in combination. Compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. In some embodiments, the present disclosure encompasses the delivery of compositions of the invention, or imaging, diagnostic, or prophylactic compositions thereof in combination with agents that improve their bioavailability, reduce and/or modify their metabolism, inhibit their excretion, and/or modify their distribution within the body.

It will further be appreciated that therapeutically, prophylactically, diagnostically, or imaging active agents utilized in combination may be administered together in a single composition or administered separately in different compositions. In general, it is expected that agents utilized in combination will be utilized at levels that do not exceed the levels at which they are utilized individually. In some embodiments, the levels utilized in combination may be lower than those utilized individually.

The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, a composition useful for treating cancer may be administered concurrently with a chemotherapeutic agent), or they may achieve different effects (e.g., control of any adverse effects).

In some embodiments, no more than 1%, no more than 2%, no more than 3%, no more than 4%, no more than 5%, no more than 6%, no more than 7%, no more than 8%, no more than 9%, no more than 10%, no more than 15%, no more than 20%, no more than 25%, no more than 30%, no more than 35%, no more than 40%, no more than 45%, or no more than 50% of cells that are not meant to be the destination of the delivery are transfected by the LNP. In some embodiments, the cells that are not meant to be the destination of the delivery are subject's non-immune cells. In some embodiments, no more than 5% of non-immune cells are transfected by the LNP. In some embodiments, the cells that are not meant to be the destination of the delivery are cells not targeted by the method. In some embodiments, the cells that are not meant to be the destination of the delivery are subject's cells not targeted by the method.

In some embodiments, the half-life of the nucleic acid delivered by the LNP described herein to the immune cell or a polypeptide encoded by the nucleic acid delivered by the LNP and expressed in the immune cell is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 2 times, at least 3 times, at least 4 times, or at least 5 times longer than the half-life of the nucleic acid delivered by a reference LNP to the immune cells or a polypeptide encoded by the nucleic acid delivered by the reference LNP and expressed in the immune cell. In some embodiments, the half-life of the nucleic acid delivered by the LNP or a polypeptide encoded by the nucleic acid delivered by the LNP is at least 10% longer than the half-life of a nucleic acid delivered by a reference LNP or a polypeptide encoded by the nucleic acid delivered by a reference LNP.

In some embodiments, the composition of the LNP differs from the composition of the reference LNP in the type of ionizable cationic lipid, relative amount of ionizable cationic lipid, length of

the lipid anchor in PEG lipid, back bone or head group of the PEG lipid, relative amount of PEG lipid, or type of immune cell targeting group, or any combination thereof. In some embodiments, the composition of the LNP differs from the composition of the reference LNP only in the type of ionizable cationic lipid. In some embodiments, the composition of the LNP differs from the composition of the reference LNP only in the amount of PEG lipid. In some embodiments, the reference LNP comprises cationic Lipid DLin-KC3-DMA, but otherwise as the same as a tested LNP. In some embodiments, the reference LNP comprises cationic Lipid DLin-KC2-DMA, but otherwise as the same as a tested LNP. In some embodiments, the reference LNP comprises cationic Lipid ALC-0315, but otherwise as the same as a tested LNP. In some embodiments, the reference LNP comprises cationic Lipid SM-102, but otherwise as the same as a tested LNP. In some embodiments, PEG lipid is a free PEG lipid.

In some embodiments, at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the immune cells are transfected by the LNP. In some embodiments, at least 10% of immune cells are transfected by the LNP. In some embodiments, the immune cells are subject's immune cells. In some embodiments, the immune cells are immune cells targeted by the method. In some embodiments, the immune cells are subject's immune cells targeted by the method.

In some embodiments, the expression level of the nucleic acid delivered by the LNP is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 2 times, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, or at least 10 times higher than the expression level of the nucleic acid delivered by a reference LNP. In some embodiments, expression level of the nucleic acid delivered by the LNP is at least 10% higher than expression level of a nucleic acid delivered by a reference LNP. In some embodiments, the expression level is measured and compared with a method described herein. In some embodiments, the expression level is measured by the ratio of cells expressing the encoded polypeptide. In some embodiments, the expression level is measured with FACS. In some embodiments, the expression level is measured by the average amount of the encoded polypeptide expressed in cells. In some embodiments, the expression

level is measured as mean fluorescence intensity. In some embodiments, the expression level is measured by the amount of the encoded polypeptide or other materials secreted by cells.

In another aspect, provided herein are methods of targeting the delivery of a nucleic acid to an immune cell of a subject. In some embodiments, the method comprises contacting the immune cell with a lipid nanoparticle (LNP). In some embodiments, the LNP comprises an ionizable cationic lipid. In some embodiments, the LNP comprises a conjugate comprising the compound of the following Formula (II):

[Lipid] – [optional linker] – [immune cell targeting group].

In some embodiments, the LNP comprises a sterol or other structural lipid. In some embodiments, the LNP comprises a neutral phospholipid. In some embodiments, the LNP comprises a free Polyethylene glycol (PEG) lipid. In some embodiments, the LNP comprises the nucleic acid.

In some embodiments, an aspect of the disclosure relates to an LNP or a pharmaceutical composition containing thereof, as disclosed herein, for use in a method of targeting the delivery of a nucleic acid to an immune cell of a subject. Such a method may be for the treatment of a disease or disorder as disclosed herein. In some embodiments, a method as disclosed herein can comprise contacting *in vitro* or *ex vivo* the immune cell of a subject with a lipid nanoparticle (LNP). In some embodiments, the LNP is an LNP as described herein in the present disclosure.

In some embodiments, the LNP provides at least one of the following benefits: (i) increased specificity of targeted delivery to the immune cell compared to a reference LNP;

(ii) increased half-life of the nucleic acid or a polypeptide encoded by the nucleic acid in the immune cell compared to a reference LNP;

(iii) increased transfection rate compared to a reference LNP; and

(iv) a low level of dye accessible mRNA (<15%) and high RNA encapsulation efficiencies, wherein at least 80% mRNA was recovered in final formulation relative to the total RNA used in LNP batch preparation.

In some aspects, provided are methods of expressing a polypeptide of interest in a targeted immune cell of a subject. In some embodiments, the method comprises contacting the immune cell with a lipid nanoparticle (LNP). In some embodiments, the LNP comprises an ionizable cationic lipid. In some embodiments, the LNP comprises a conjugate comprising the following structure of Formula (II):

[Lipid] – [optional linker] – [immune cell targeting group].

In some embodiments, the LNP comprises a sterol or other structural lipid. In some embodiments, the LNP comprises a neutral phospholipid. In some embodiments, the LNP comprises a free Polyethylene glycol (PEG) lipid. In some embodiments, the LNP comprises a nucleic acid encoding the polypeptide. In some embodiments, an aspect of the disclosure relates to an LNP or a pharmaceutical composition containing thereof, as disclosed herein, for use in a method of expressing a polypeptide of interest in a targeted immune cell of a subject. Such a method may be for the treatment of a disease or disorder as disclosed herein. In some embodiments, a method as disclosed herein can comprise contacting *in vitro* or *ex vivo* the immune cell of a subject with a lipid nanoparticle (LNP).

In some embodiments, the LNP provides at least one of the following benefits: (i) increased expression level in the immune cell compared to a reference LNP;

(ii) increased specificity of expression in the immune cell compared to a reference LNP; (iii) increased half-life of the nucleic acid or a polypeptide encoded by the nucleic acid in the immune cell compared to a reference LNP;

(iv) increased transfection rate compared to a reference LNP; and

(v) a low level of dye accessible mRNA (<15%) and high RNA encapsulation efficiencies, wherein at least 80% mRNA was recovered in final formulation relative to the total RNA used in LNP batch preparation.

In some aspects, provided are methods of modulating cellular function of a target immune cell of a subject. In some embodiments, the method comprises administering to the subject a lipid nanoparticle (LNP). In some embodiments, the LNP comprises an ionizable cationic lipid. In

some embodiments, the LNP comprises a conjugate comprising the following structure of Formula (II):

[Lipid] – [optional linker] – [immune cell targeting group].

In some embodiments, the LNP comprises a sterol or other structural lipid. In some embodiments, the LNP comprises a neutral phospholipid. In some embodiments, the LNP comprises a free Polyethylene glycol (PEG) lipid. In some embodiments, the LNP comprises a nucleic acid encoding a polypeptide for modulating the cellular function of the immune cell. In some embodiments, an aspect of the disclosure relates to an LNP or a pharmaceutical composition containing thereof, as disclosed herein, for use in a method of modulating cellular function of a targeted immune cell of a subject. Such a method may be for the treatment of a disease or disorder as disclosed herein. In some embodiments, a method as disclosed herein can comprise contacting *in vitro* or *ex vivo* the immune cell of a subject with a lipid nanoparticle (LNP).

In some embodiments, the LNP provides at least one of the following benefits: (i) increased expression level in the immune cell compared to a reference LNP;

(ii) increased specificity of expression in the immune cell compared to a reference LNP;

(iii) increased half-life of the nucleic acid or a polypeptide encoded by the nucleic acid in the immune cell compared to a reference LNP;

(iv) increased transfection rate compared to a reference LNP;

(v) the LNP can be administered at a lower dose compared to a reference LNP to reach the same biologic effect in the immune cell; and

(vi) a low level of dye accessible mRNA (<15%) and high RNA encapsulation efficiencies, wherein at least 80% mRNA was recovered in final formulation relative to the total RNA used in LNP batch preparation.

In some embodiments, the modulation of cell function comprises reprogramming the immune cells to initiate an immune response. In some embodiments, the modulation of cell function comprises modulating antigen specificity of the immune cell.

In some aspects, provided are methods of treating, ameliorating, or preventing a symptom of a disorder or disease in a subject in need thereof. In any of the embodiments described herein concerning a method of treating, ameliorating, and/or preventing a symptom of a disorder or disease by administration of, e.g., a LNP of the invention, it is intended that said disclosures are also interpreted as providing, e.g., the LNP for use in said methods of treating, ameliorating, and/or preventing a symptom of a disorder or disease. In some embodiments, the method comprises administering to the subject a lipid nanoparticle (LNP) for delivering a nucleic acid into an immune cell of the subject. In some embodiments, the LNP comprises an ionizable cationic lipid. In some embodiments, the LNP comprises a conjugate comprising the following structure of Formula (II):

[Lipid] – [optional linker] – [immune cell targeting group].

In some embodiments, the LNP comprises a sterol or other structural lipid. In some embodiments, the LNP comprises a neutral phospholipid. In some embodiments, the LNP comprises a free Polyethylene glycol (PEG) lipid. In some embodiments, the LNP comprises the nucleic acid.

In some embodiments, the nucleic acid modulates the immune response of the immune cell, therefore to treat or ameliorate the symptom. In some embodiments, an aspect of the disclosure relates to an LNP or a pharmaceutical composition containing thereof, as disclosed herein, for use in a method of treating, ameliorating, or preventing a symptom of a disorder or disease in a subject in need thereof. A disease or disorder may be as disclosed herein. In some embodiments, a method as disclosed herein can comprise contacting in vitro or ex vivo the immune cell of a subject with a lipid nanoparticle (LNP).

In some embodiments, the LNP provides at least one of the following benefits: (i) increased specificity of delivery of the nucleic acid into the immune cell compared to a reference LNP;

(ii) increased half-life of the nucleic acid or a polypeptide encoded by the nucleic acid in the immune cell compared to a reference LNP;

(iii) increased transfection rate compared to a reference LNP;

(iv) the LNP can be administered at a lower dose compared to a reference LNP to reach the same treatment efficacy;

(v) increased level of gain of function by an immune cell compared to a reference LNP; and

(vi) a low level of dye accessible mRNA (<15%) and high RNA encapsulation efficiencies, wherein at least 80% mRNA was recovered in final formulation relative to the total RNA used in LNP batch preparation.

In some embodiments, the disorder is an immune disorder, an inflammatory disorder, or a cancer. In some embodiments, the nucleic acid encodes an antigen for use in a therapeutic or prophylactic vaccine for treating or preventing an infection by a pathogen.

In some embodiments, no more than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% of non-immune cells are transfected by the LNP. In some embodiments, no more than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% of undesired immune cells that are not meant to be the destination of the delivery are transfected by the LNP. In some embodiments, the half-life of the nucleic acid delivered by the LNP to the immune cell or a polypeptide encoded by the nucleic acid delivered by the LNP is at least 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 1.5 times, 2 times, 3 times, 4 times, 5 times, 10 times, or longer than the half-life of nucleic acid delivered by a reference LNP to the immune cell or a polypeptide encoded by the nucleic acid delivered by the reference LNP.

In some embodiments, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more immune cells that are meant to be the destination of the delivery are transfected by the LNP.

In some embodiments, expression level of the nucleic acid delivered by the LNP is at least 5%, at least 10%, 1.5 time, 2 times, 3 times, 4 times, 5 times, 10 times, 15 times, 20 times or more

higher than expression level of nucleic acid in the same immune cells delivered by a reference LNP.

In one aspect, provided are lipid nanoparticles (LNPs) for delivering a nucleic acid into NK cells of the subject. The LNP comprises:

(a) An ionizable cationic lipid, (b) A conjugate comprising the following structure of Formula (II):

[Lipid] – [optional linker] – [immune cell targeting group];

(c) A sterol or other structural lipid;

(d) A neutral phospholipid;

(e) A free Polyethylene glycol (PEG) lipid; and

(f) the nucleic acid. In some embodiments, the immune cell targeting group comprises an antibody that binds CD56.

In one aspect, provided are lipid nanoparticles (LNPs) for delivering a nucleic acid into immune cells of the subject. The LNP comprises:

(a) An ionizable cationic lipid;

(b) A conjugate comprising the following structure: [Lipid] – [optional linker] – [immune cell targeting group];

(c) A sterol or other structural lipid;

(d) A neutral phospholipid;

(e) A free Polyethylene glycol (PEG) lipid; and

(f) the nucleic acid.

In some embodiments, the immune cell targeting group comprises an antibody that binds CD7 or CD8, and the free PEG lipid is DMG-PEG or DPG-PEG.

In some aspects, provided are methods of targeting the delivery of a nucleic acid to an immune cell of a subject. In some embodiments, the method comprises contacting the immune cell with a lipid nanoparticle (LNP) provided herein. In some embodiments, the method is for targeting NK cells. In some embodiments, the immune cell targeting group binds to CD56. In some embodiments, the method is for targeting both T cells and NK cells simultaneously. In some embodiments, the immune cell targeting group binds to CD7, CD8, or both CD7 and CD8. In some embodiments, the method is for targeting both CD4+ and CD8+ T cells simultaneously. In some embodiments, the immune cell targeting group comprises a polypeptide that binds to CD3 or CD7.

In some aspects, provided are methods of expressing a polypeptide of interest in a targeted immune cell of a subject. In some embodiments, the method comprises contacting the immune cell with a lipid nanoparticle (LNP) provided herein.

In some aspect, provided are method of modulating cellular function of a target immune cell of a subject. In some embodiments, the method comprises administering to the subject a lipid nanoparticle (LNP) provided herein.

In some aspects, provided are method of treating, ameliorating, or preventing a symptom of a disorder or disease in a subject in need thereof. In some embodiments, the method comprises administering to the subject a lipid nanoparticle (LNP) provided herein.

In some aspects, provided are methods of treating a disease or disorder related to CD8 in a subject. In some embodiments, the method comprises administering a pharmaceutical composition described herein to the subject. In some embodiments, the disease or disorder is cancer.

LNPs disclosed in the present disclosure and as claimed are suitable for the methods described above.

VIII. KITS FOR USE IN MEDICAL APPLICATIONS

Another aspect of the invention provides a kit for treating a disorder. The kit comprises: an ionizable cationic lipid, a lipid-immune cell targeting group conjugate, a lipid nanoparticle composition comprising an ionizable cationic lipid and/or a lipid-immune cell targeting group conjugate with or without an encapsulated payload (e.g., an mRNA), and instructions for treating a medical disorder, such as, cancer or a microbial or viral infection.

EXAMPLES

The present disclosure exemplifies compositions, preparations, formulations, nanoparticles, and/or nanomaterials described herein. The present disclosure also exemplifies methods of preparing, characterizing, and validating compositions, preparations, formulations, nanoparticles, and/or nanomaterials described herein.

Example 1: Materials and Methods

The present Example provides exemplary materials and methods of preparing, characterizing, and validating compositions, preparations, nanoparticles, and/or nanomaterials described herein.

LNP Preparations

Among other things, the present Example provides for exemplary LNP preparations.

Lipid nanoparticle components are dissolved in 100% ethanol at specified lipid component molar ratios. Nucleic acid (NA) cargo is dissolved in 10 mM citrate, 100 mM NaCl, pH 4.0, resulting in a concentration of NA cargo of approximately 0.22 mg/mL. In some embodiments, NA cargos include both a functional NA and a reporter DNA barcode mixed at mass ratios of 1:10 to 10:1 functional NA to barcode. As described herein, a NA can be a siRNA, an anti-sense, an expressing DNA, or mRNA.

LNPs are prepared with a total lipid to NA mass ratio of 11.7. LNPs are formed by microfluidic mixing ofthe lipid and NA solutions using a Precision Nanosystems NanoAssemblr Spark or Benchtop series Instruments, according to the manufacturers protocol. A ratio of aqueous to organic solvent of approximately 2:1 or 3:1 is maintained during mixing using differential flow rates. After mixing, LNPs are collected, diluted in PBS (approximately 1:1 v/v). Further buffer

exchange is conducted using dialysis in PBS at 4°C for 4 to 24 hours against a 20kDa filter. After this initial dialysis, each individual LNP preparation is characterized via dynamic light scattering (DLS) to measure the size (e.g., diameter) and polydispersity. In addition, pKa of a subpopulation of LNPs is measured via a 2-(p-toluidino)-6-napthalene sulfonic acid (TNS) assay. LNPs falling within specific diameter and polydispersity ranges are pooled, and further dialyzed against phosphate buffer saline (PBS) at 4°C for 1 to 4 hours against a 100 kDa dialysis cassette. After the second dialysis, LNPs are sterile filtered using 0.22μM filter and stored at 4°C for further use.

LNP characterization

DLS - LNP hydrodynamic diameter and polydispersity index (PDI) are measured using high throughput dynamic light scattering (DLS) (DynaPro plate reader II, Wyatt). LNPs are diluted IX PBS to an appropriate concentration and analyzed. Concentration of NA is determined by Qubit microRNA kit (for siRNA) or HS RNA kit (for mRNA) per manufacturer's instructions. Encapsulation efficiency is determined by measuring nucleic acid concentration in unlysed and lysed LNPs.

pKa

A stock solution of 10 mM HEPES (Sigma Aldrich), 10 mM MES (Sigma Aldrich), 10 mM sodium acetate (Sigma), and 140 nM sodium chloride (Sigma Aldrich) is prepared, and pH is adjusted using hydrogen chloride and sodium hydroxide to a range of about pH 4-10. Elsing four replicates for each pH value, 140 pL pH-adjusted buffer is added to a 96-well plate, followed by the addition 5 pL of 2-(p-toluidino)-6- napthalene sulfonic acid (60 pg/ mL). 5pL of LNP is added to each well. After 5 min of incubation under gentle shaking, fluorescence is measured using an excitation wavelength of 325 nm and emission wavelength of 435 nm (BioTek Synergy H4 Hybrid).

LNP Administration

Male and female mice aged approximately 8-12 weeks are used for the studies described by the present Example. Each mouse is temporarily restrained, and pooled LNP is administered intravenously (IV) via tail vein injection in up to five animals per experiment. Agematched mice are also used to administer vehicle (IX PBS) via tail vein injection in up to three animals per experiment. At 72 hours post-dose, tissues including liver, spleen, bone marrow, kidney, lung, muscle, and blood are collected for analysis.

Flow

Liver, kidney, lung, and muscle tissues are mechanically, and then enzymatically digested using a mixture of proteinases, then passed through a 70uM filter to generate single cell suspensions. Spleen tissues are mechanically digested to generate single cell suspensions. All tissues are treated with (Ammonium-Chloride-Potassium) ACK buffer to lyse red blood cells, and then stained with fluorescently-labeled antibodies for flow cytometry and fluorescence-activated cell sorting (FACS). Commercially available antibodies are used. Using a BD FACSMelody (Becton Dickinson), samples are acquired via flow cytometry to generate gates prior to sorting. In general, gating structure is size -> singlet cells -> live cells -> cells of interest. T cells are defined as CD45+CD3+, monocytes are defined as CD45+CDllb+, and B cells are defined as CD45+CD19+. Endothelial cells are defined as CD31+, monocytes and Kupffer cells as CD45+CDllb+ and hepatocytes as CD31-/CD45-. For siRNA studies, downregulation of the target gene is gated. For mRNA studies, upregulation of the target gene is gated. Tissues from vehicle-dosed mice are used to set the gates for sorting. Up to 1 million cells of each cell subset with the correct phenotype are sorted into PBS. After sorting, cells are pelleted via centrifugation and DNA is extracted using Quick Extract DNA Extraction Solution (Lucigen) according to manufacturer's protocol. Following DNA extraction, DNA is stored at -20°C.

Concentration & Encapsulation Efficiency

Concentration of NA is determined by Qubit microRNA kit (for siRNA) or HS RNA kit (for mRNA) per manufacturer's instructions. Encapsulation efficiency is determined by measuring unlysed and lysed LNPs.

hEPO Expression

For human EPO (hEPO) protein expression, mice are temporarily restrained and bled at 6 hours post-administration (via tail vein). Blood is collected in heparin tubes, processed to plasma, and stored at -80°C until ready to use. Appropriate dilutions of plasma is used to measure hEPO protein using R&D systems ELISA kit (DuoSet; DY286-05) according to manufacturer's instructions.

Tolerability ALT / AST Quantification

For rat Aspartate Transaminase (AST) and Alanine Transaminase (ALT) quantification, rats are temporarily restrained and bled at 2, 4, 6, 24, 48, and 72 hrs hours post-administration. Blood is collected in heparin tubes, processed to plasma, and stored at -80°C until ready to use. AST is quantified using AST/GOT reagent (ThermoFisher, TR70121) and ALT is quantified using ALT/GPT reagent (ThermoFisher, TR71121) according to manufacturer's instructions.

RatMCP-I ELISA

For Rat Monocype Chemoattractant Protein-1 (MCP-I) protein expression, rats are temporarily restrained and bled at 2, 4, 6, 24, 48, and 72 hrs hours post-administration. Blood is collected in heparin tubes, processed to plasma, and stored at -80°C until ready to use. Appropriate dilutions of plasma are used to measure MCP-I protein using R&D systems ELISA kit (DuoSet; DY3144-05) according to manufacturer's instructions.

Example 2: Potency

Exemplary LNP preparations with potent delivery to various cell types may be assayed as described in this Example.

The present Example provides exemplary LNP compositions, preparations, nanoparticles, and/or nanomaterials for potent delivery to various cell types described herein.

Based on the results derived from Screen, several lipids exemplary LNP preparations are identified for use in splenic delivery, and in particular, for use in B cell delivery. These identified lipids are formulated into LNP preparations and screened using a Cre reporter system. Three Ai 14 mice are used per group. Payloads comprise 0.3 mg/kg Cre mRNA. Data is collected at 168 hours post-injection. Results are compared to an ALC-315 LNP preparation as a control.

Example 3: Preparation of lipid nanoparticle composition

LNP compositions were prepared to result in an ionizable lipid: structural lipid: sterol lipid: PEG-Lipid at a molar ratio of 33:39.9:25.4:1.5 respectively. Lipids were mixed at the following ratios and dissolved in ethanol (organic phase). The mRNA phase (aqueous phase) was prepared using RNA-ase free water and 500 mM Citrate pH 4 buffer to reach a final concentration of 10 mM citrate and 0.12 mg/ml of mRNA.

Each of the LNP composition, the lipids and mRNA were mixed at a 3:1 ratio by volume on the NanoAssemblr Ignite at a flow rate of 12 ml/min. The samples were loaded into a Slide-a-Lyzer G3 Dialysis Cassette (10k MWCO) and dialyzed into 200 times the sample volume of 1x TBS diluted from 20x stock (ThermoFischer Scientific) for 2 hours at room temperature. The dialysis buffer was then switched for fresh 1x TBS buffer and dialyzed overnight for at least 12 hours at 4C. The dialysis samples were then collected and concentrated using Amicon Ultra Centrifugation filters (100k MWCO) at 2000g. The concentrated samples were then sterile filtered using a 0.2um mPES filter. The samples then undergo analytics including size and polydispersity (Malvern Panalytical) and mRNA for encapsulation using Ribogreen RNA assay kit (ThermoFischer Scientific)

Example 4: Targeted LNP formulation Protocol

LNP compositions were prepared to result in an ionizable lipid: structural lipid: sterol lipid: PEG-Lipid: DSPE PEG FAB:DSPE PEG Azide at a molar ratio of 33:39.9:25.4:1.5:0.05:0.1 respectively. The mRNA was suspended in a 10mM citrate pH 4 buffer (aqueous phase). The ionizable lipid, structural lipid, and cholesterol were dissolved in the ethanol stream (organic

phase). The DSPE PEG Fab with small amounts of DSPE PEG Azide and DMG PEG 2000 were dissolved in 1x TBS in the dilution stream (Dilution phase). The LNPs were then mixed at a 3:1:4 ratio of aqueous phase to organic phase to dilution phase.

Example 5: Ribogreen for LNP Analytics

The mRNA used in the formulation was diluted using TE buffer to 2 μg/mL and then serial diluted to 1μg/mL, 0.5 μg/mL, 0.25 μg/mL, 0.125 μg/mL. Two standard curves were produced, one in TE buffer and one in 4% Triton buffer (TX). LNPs that have been concentrated and sterile filtered were diluted twice using TE to fit within the TE and TX standard curve. The samples were loaded onto a 96 well plate and TE or 4% Triton buffer was added to the well. Ribogreen dye was diluted 1:200 times with TE buffer and then added to all wells. The plate was then incubated and shaken on the plate reader at 37 for 3 mins before reading on the plate reader.

Example 6: Flow protocol

8-10 week old C57BL/6 animals (Stock no: 000664) were acquired from JAX and allowed to acclimate for a minimum period of 3 days. Lipid nanoparticle (LNP) formulations containing eGFP mRNA at a range of doses was administered intravenously at a dose volume of 10uL/gram of body weight. 16-hours following dosing, animals were euthanized, and terminal blood and spleen were collected and processed for flow cytometry.

Flow data was analyzed using FlowJo and each sample was gated for singlets, live cells and T cell subpopulations (CD4 and CD8). GFP expression was calculated for each experimental group and GFP+ cells were reported as a percentage of total T cells, T cell sub-types and Non T cells. GFP expression was also calculated on all live cells along with MFI across different cell populations.

Example 7: Targeted Lipids

Associating a targeting agent (e.g., Ab, scFv or Fab) to targeting LNP functionalized the surface of the LNP. A click handle on the targeting agent complementary to a click chemistry present on the LNP is activated according to methods known in the art. For example, a LNP comprising 1,2-

distearoyl-sn-glycero-3-phosphoethanolamine-PEG-tetrazine (DSPE-PEG-Tz) is associated with a targeting agent modified specifically or nonspecifically with the complementary trans-cyclooctene (TCO) click handle. 1 μM targeting molecule (e.g., anti-CD5 Fab, scFV or Ab or anti-CD8 Fab, scFv or Ab) is added to the LNP, and the click chemistry association is performed according to methods known in the art at room temperature for 16 hours. Unassociated antibodies are removed by passing the LNP through a size-exclusion chromatograph (SEC) column using Sephacryl 400 resin.

SPAAC Conjugation:

Fab'-DBCO:

Fab' were conjugated to DBCO-PEG_3-maleimide via covalent coupling between the maleimide group and a C-terminal cysteine in the light chain (LC). The protein (5-7 mg/mL in sodium acetate buffer, pH 5.5), was neutralized by 1 M sodium bicarbonate buffer, pH 9.0, followed by addition of 1.2 molar equivalents of DBCO-PEG_3-Mal for 2 hours at room temperature. The excess of DBCO-PEG_3-Mal was removed by PD-10 desalting column.

DSPE-PEG2000-Fab:

The conjugation reaction was carried out using 3 molar equivalents of DBCO-PEG_3-N_3 (Avanti Polar Lipids) and Fab-DBCO in pH 7.4 phosphate buffer at room temperature for 12 - 16 hours. The production of the resulting conjugate was monitored by SDS-PAGE and LC-MS. The resulting conjugate was isolated using a 100 kDa Millipore regenerated cellulose membrane filtration using pH 7.4 phosphate buffer and stored at 4°C prior to use.

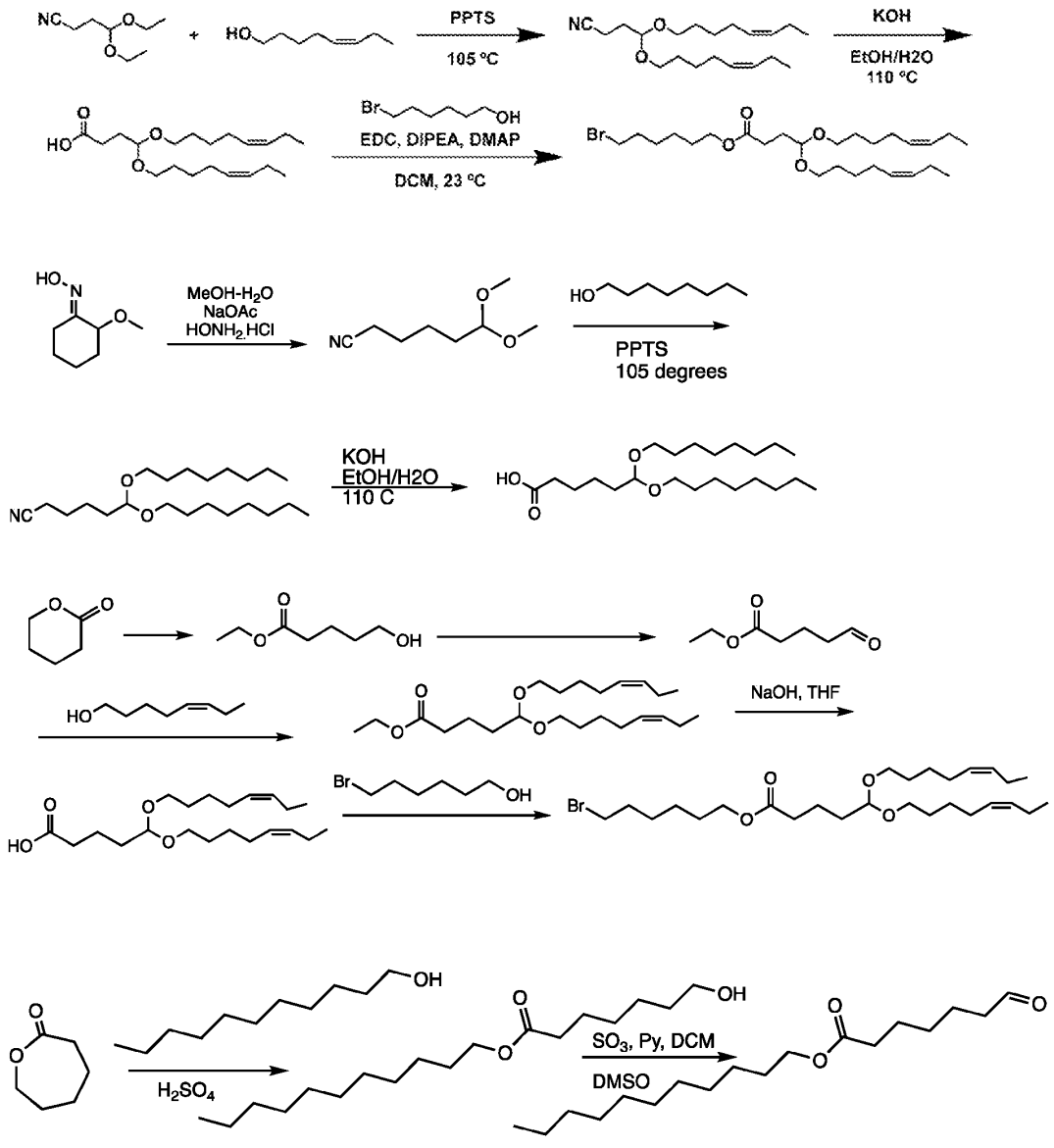
Example 8: Synthesis of Ionizable lipids

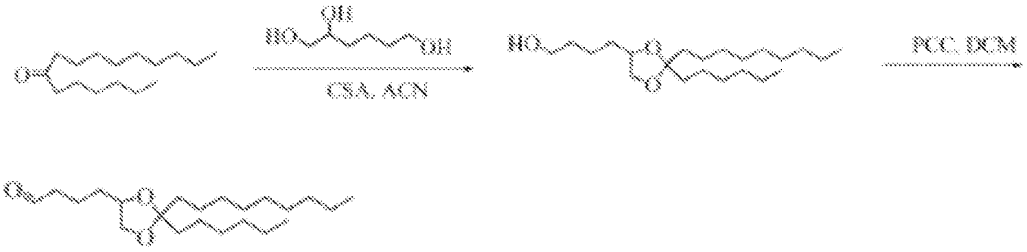
The present Example provides exemplary materials and methods of preparing, characterizing, and validating ionizable lipids as described herein. As described in the Examples below, in certain exemplary embodiments, compounds are prepared according to the following general procedures. It will be appreciated that, although the general methods depict the synthesis of certain compounds of the present disclosure, the following general methods and other methods

known to one of ordinary skill in the art can be applied to all compounds and subclasses and species of each of these compounds, as described herein.

The following example lipids are prepared according to the below synthetic schemes.

Scheme 1 (Intermediates)

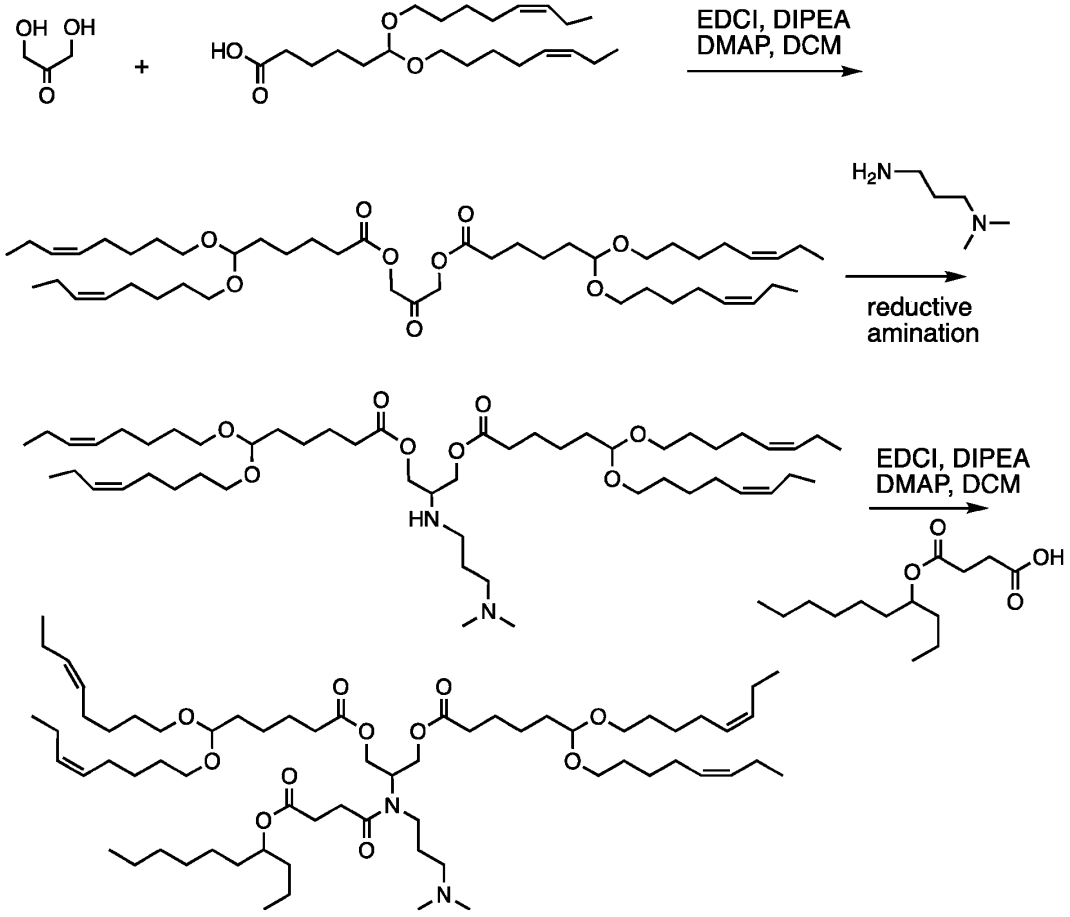




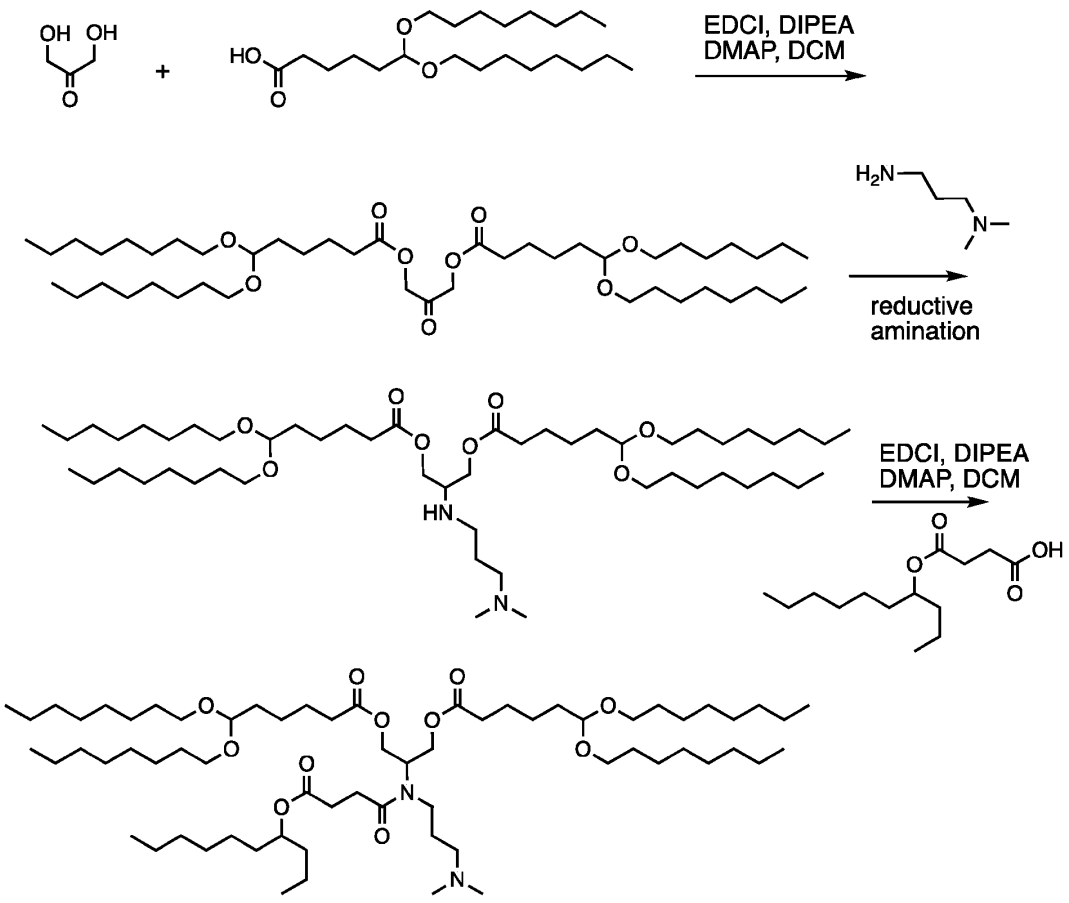
5

Scheme 2 – Representative syntheses of class A compounds

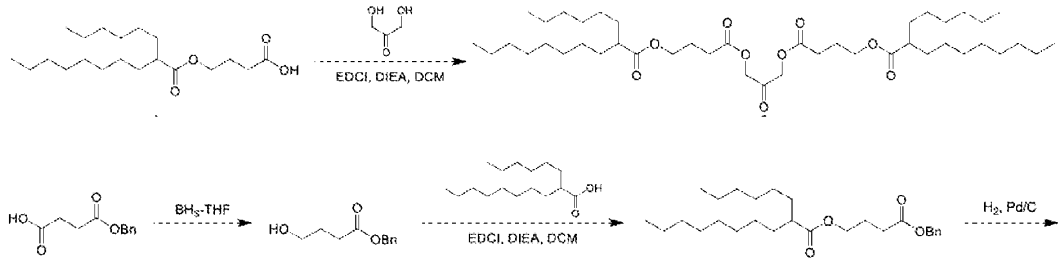
Lipid A-4

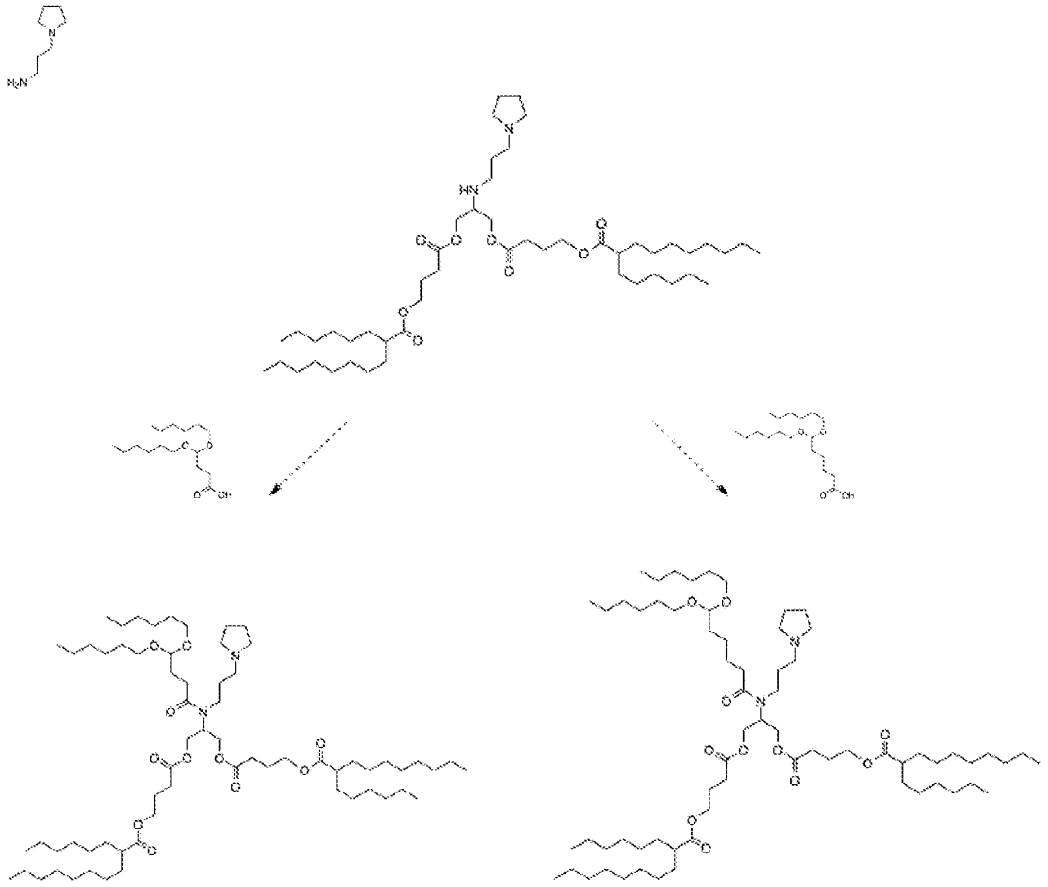


Lipid A-31



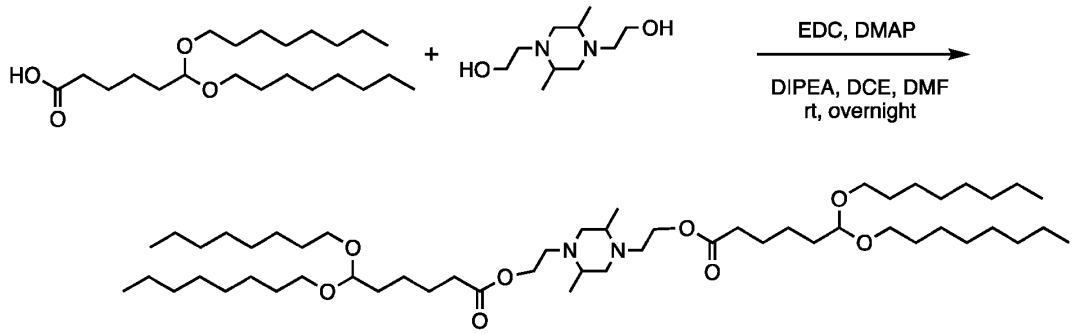
Scheme 2a



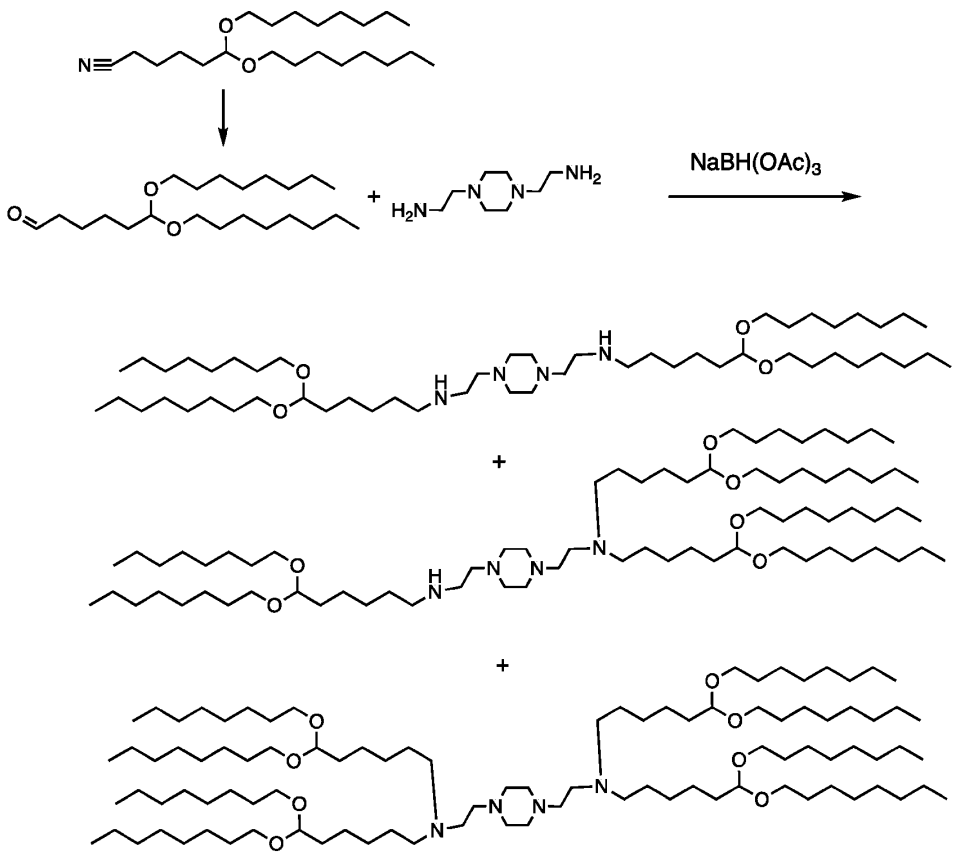


Scheme 3 – Representative syntheses of class B compounds

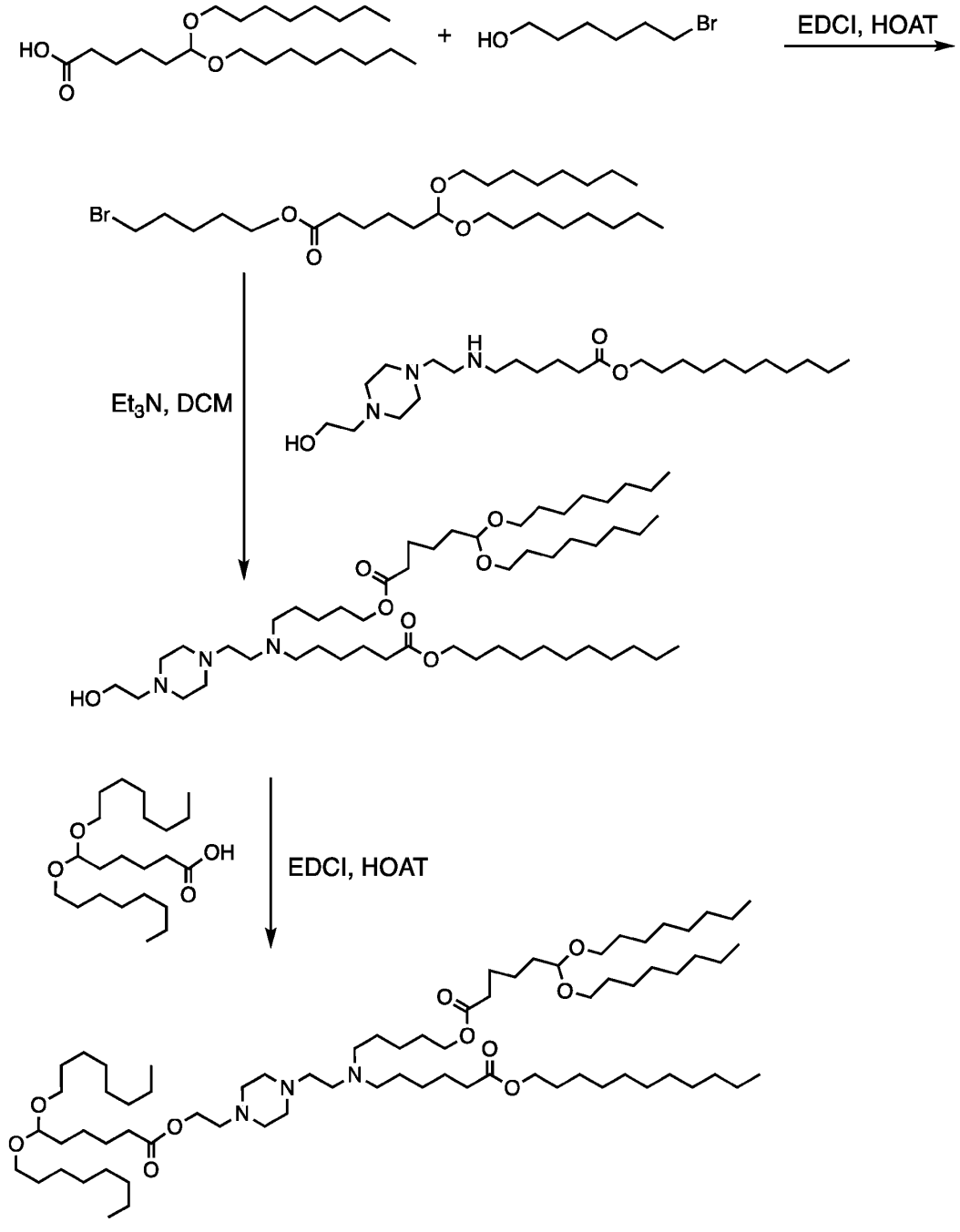
Lipid B-9



Lipids B-11, B-12 and B-13

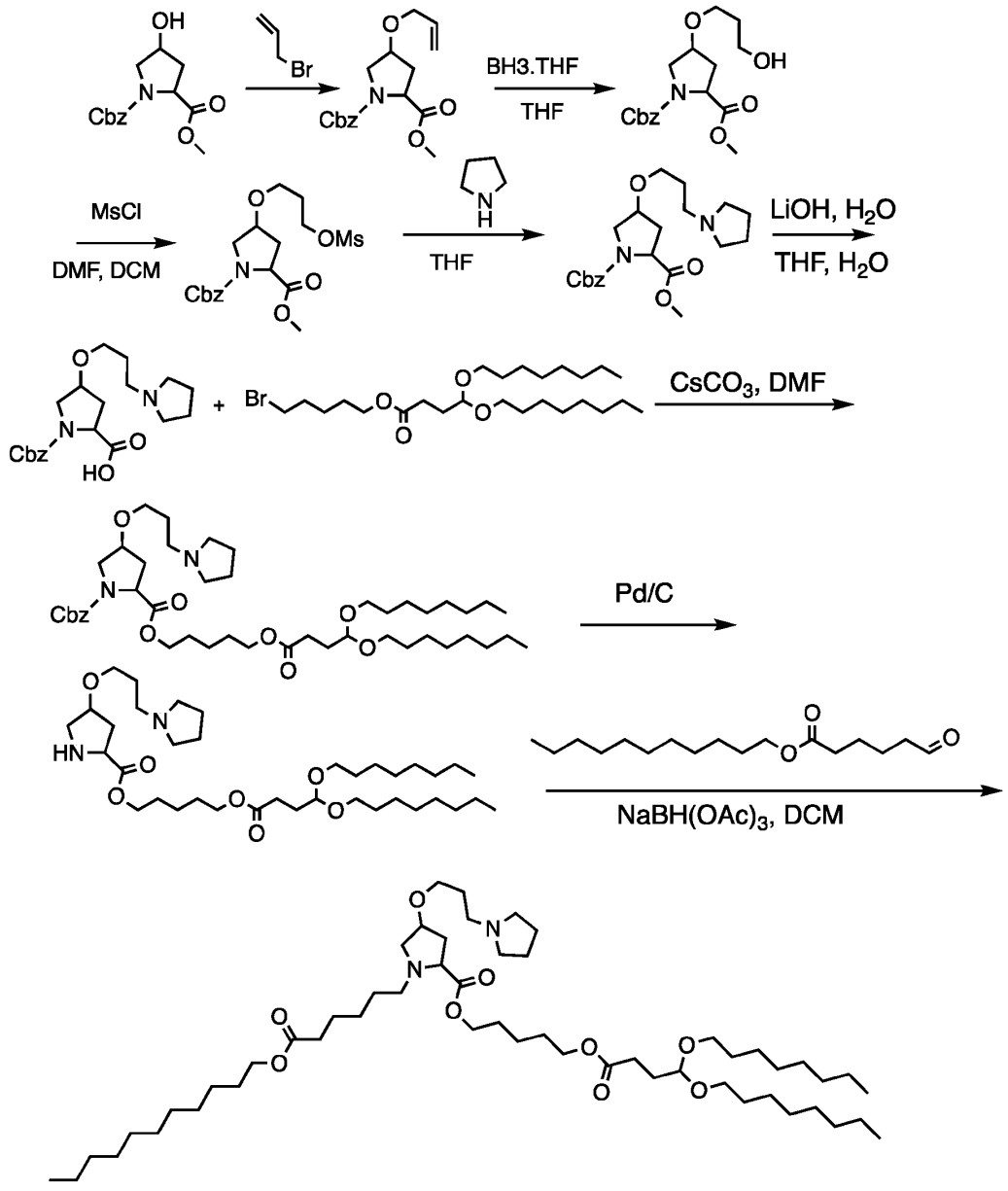


Lipid B-10

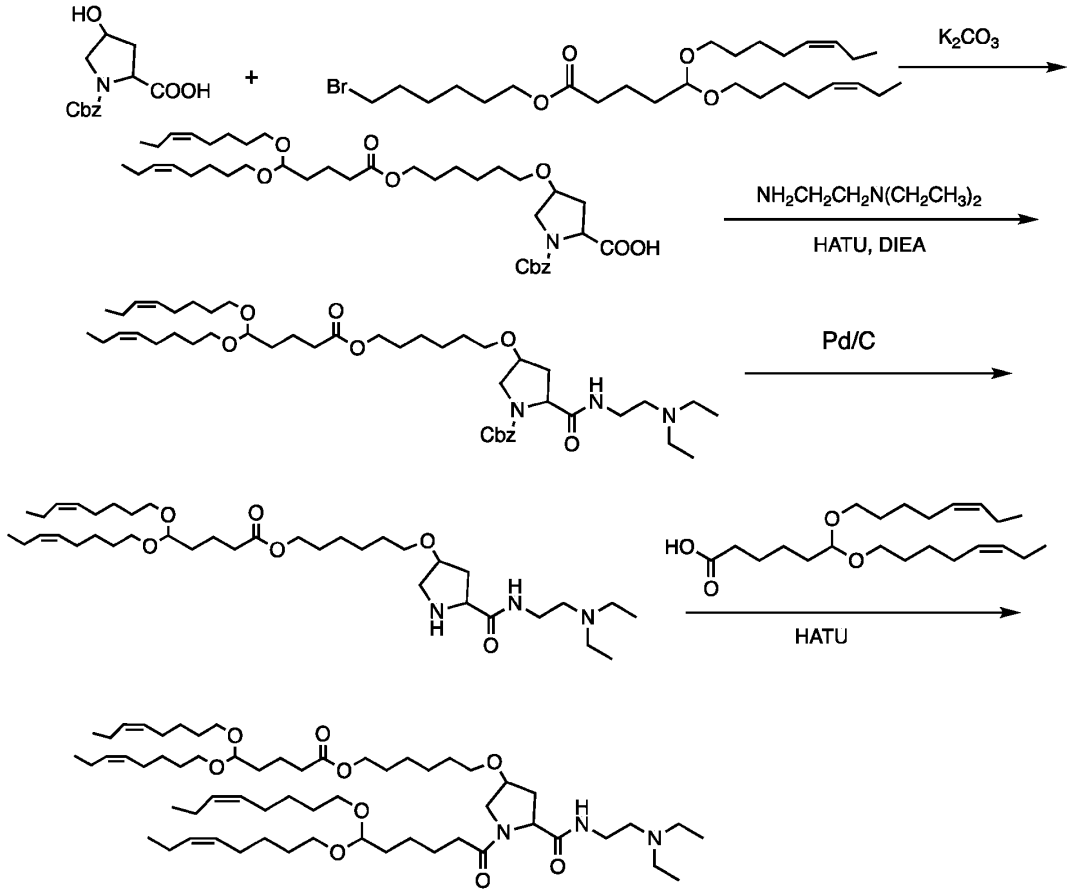


Scheme 4 – Representative syntheses of class C compounds

Lipid C-15

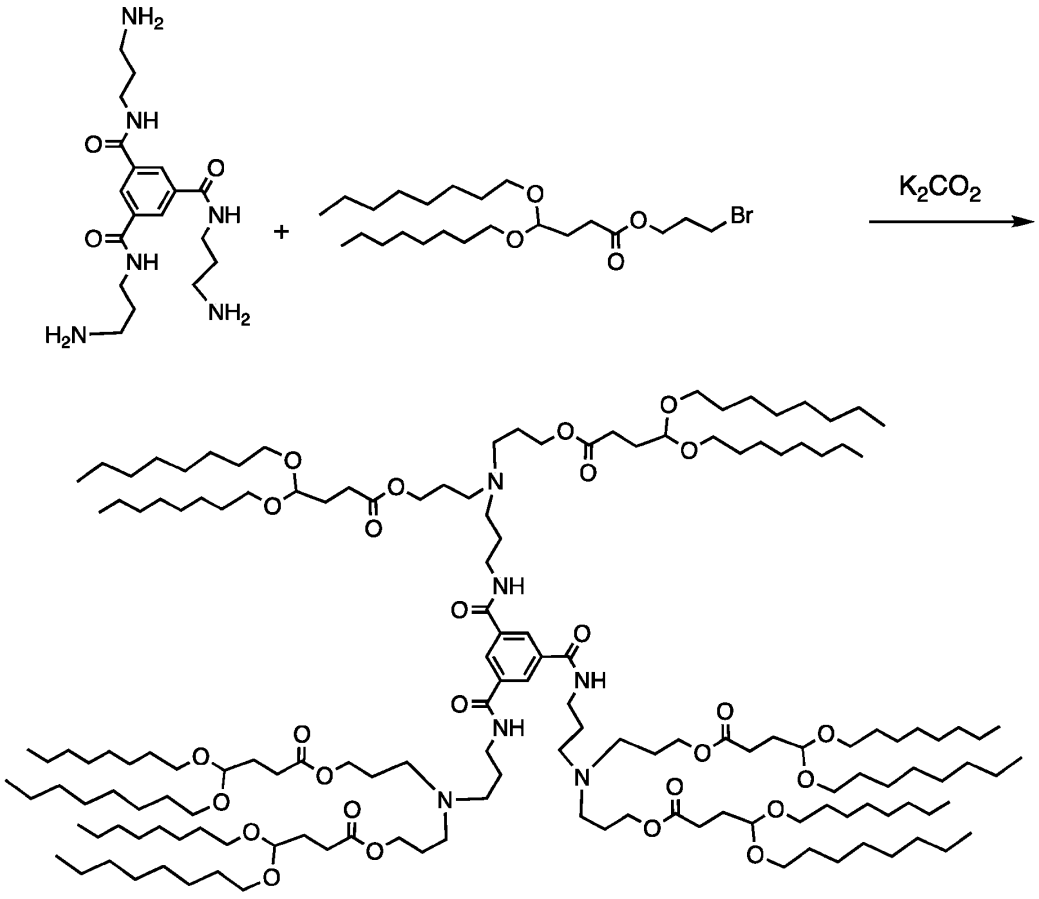


Lipid C-26


HO
N
Cbz
COOH
+
Br
O
O
O
O
O
O
O
O
K_2CO_3
$NH_2CH_2CH_2N(CH_2CH_3)_2$
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HATU
HO
H
N

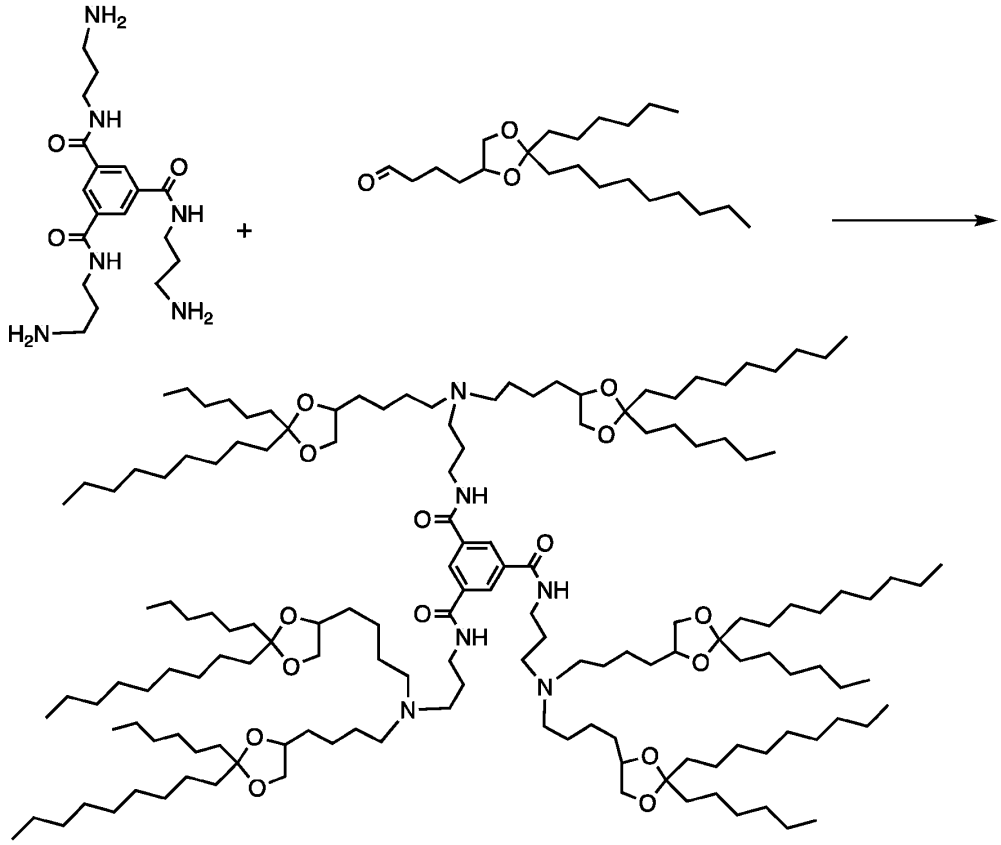
Scheme 5 – Representative syntheses of class E compounds

Lipid E-1



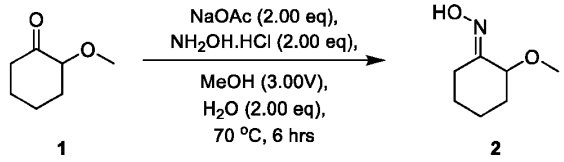
+ other mixtures

Lipid E-3



+ other mixtures

General procedure for preparation of compound 2



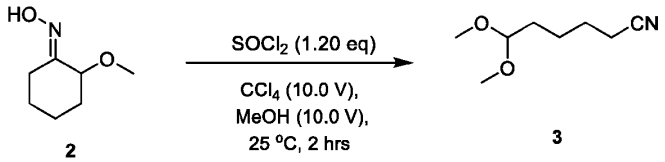
To a solution of compound **1** (100 g, 780 mmol) in MeOH (300 mL) and H_2O (600 mL) was added NaOAc (128 g, 1.56 mol) and $NH_2OH.HCl$ (108 g, 1.56 mol). The mixture was stirred at 70 °C for 6 hrs. TLC (Petroleum ether/Ethyl acetate = 3/1, product: R_f = 0.49, starting material: R_f = 0.59) showed the starting material was consumed completely. The reaction mixture was

concentrated under reduced pressure to remove MeOH. The residue was diluted with H_2O (500 mL) and extracted with EtOAc (300 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate=10/1 to 1/1). The crude product was used into the next step without further purification. Compound **2** (108 g, crude) was obtained as a yellow oil.

^{1}H NMR: ET89868-12-P1A1 (400 MHz, DMSO-d_6)

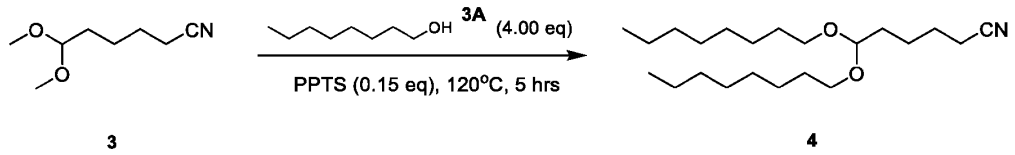
δ = 10.61 (s, 1H), 3.62 (s, 1H), 3.11 (s, 3H), 2.97 (br d, J = 14.3 Hz, 1H), 1.99 (s, 1H), 1.59 - 1.77 (m, 3H), 1.48 (br s, 2H), 1.25 (br s, 1H)

General procedure for preparation of compound 3



To a solution of compound **2** (108 g, 754 mmol) in CCl_4 (1.00 L) was added $SOCl_2$ (108g, 905mmol) and MeOH (1.00 L). The mixture was stirred at 0-5 °C for 2 hrs. TLC (Petroleum ether/Ethyl acetate = 3/1, product: R_f = 0.55, starting material: R_f = 0.59) showed the starting material was consumed completely. The reaction mixture was diluted with EtOAc 3.00 L and washed with brine 2.00 L, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. Compound **3** (107 g, crude) was obtained as a brown oil.

General procedure for preparation of compound 4

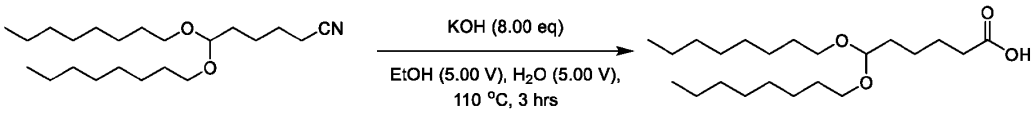


To a solution of compound **3** (40.0 g, 254 mmol) in compound **3A** (133 g, 1.01 mol) was added PPTS (9.60 g, 38.2 mmol). The mixture was stirred at 120 °C for 5 hrs. TLC (Petroleum ether/Ethyl acetate = 3/1, product: R_f = 0.63, starting material: R_f = 0.28) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate=200/1 to 80/1). Compound **4** (30.0 g, 84.9 mmol, 33.4% yield) was obtained as colourless oil.

^{1}H NMR: ET89868-26-P1A1 (400 MHz, $CDCl_3$)

δ = 4.46 (s, 1H), 3.56 (s, 2H), 3.42 (s, 2H), 2.35 (s, 2H), 1.56 (br d, J = 7.0 Hz, 10H), 1.24 - 1.37 (m, 20H), 0.89 (s, 6H)

General procedure for preparation of compound 5



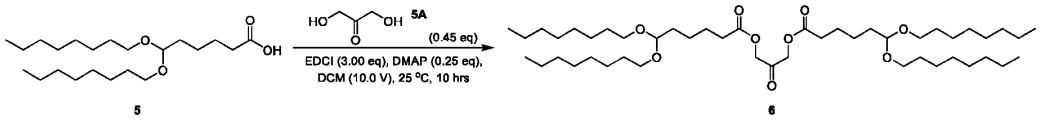
4 **5**

To a solution of compound **4** (30.0 g, 84.9 mmol) in EtOH (150 mL) and H_2O (150 mL) was added KOH (38.1 g, 679 mmol). The mixture was stirred at 110 °C for 5 hrs. TLC (Petroleum ether/Ethyl acetate = 3/1, product: R_f = 0.53, starting material: R_f = 0.76) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove EtOH. The residue was diluted with H_2O 300 mL and acidified to PH = 5 using 1M HCl. Then, the mixture was extracted with EtOAc 900 mL (300 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. Compound **5** (31.6 g, crude) was obtained as a yellow oil.

^{1}H NMR: ET89868-32-P1B1 (400 MHz, DMSO-d_6)

δ = 4.39 (br s, 1H), 3.27 - 3.52 (m, 4H), 2.10 (br s, 2H), 1.46 (br s, 8H), 1.25 (br s, 22H), 0.85 (br s, 6H)

General procedure for preparation of compound 6

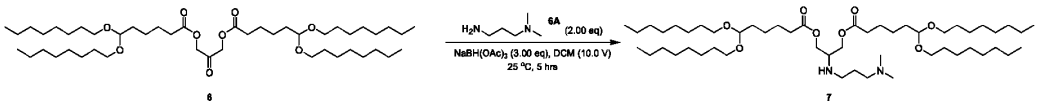


To a solution of compound **5** (31.6 g, 84.8 mmol) in DCM (300 mL) was added compound **5A** (3.47 g, 38.6 mmol), EDCI (22.2 g, 116 mmol) and DMAP (1.18 g, 9.64 mmol). The mixture was stirred at 25 °C for 10 hrs. TLC (Petroleum ether/Ethyl acetate = 3/1, product: R_f = 0.63, starting material: R_f = 0.51) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove DCM. The residue was washed with H_2O 200 mL, and then extracted with Petroleum ether 900 mL (300 mL x 3). The combined organic layers were washed with brine 200 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. Compound **6** (33.0 g, crude) was obtained as a yellow oil.

^{1}H NMR: ET89868-35-P1A2 (400 MHz, $CDCl_3$)

δ = 4.75 (s, 4H), 4.46 (s, 2H), 3.55 (s, 4H), 3.41 (s, 4H), 2.44 (s, 4H), 1.56 (br s, 18H), 1.28 (br s, 42H), 0.89 (s, 12H)

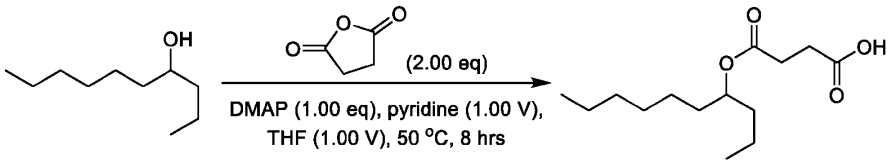
General procedure for preparation of compound 7



To a solution of compound **6** (8.00 g, 10.0 mmol) in DCM (80.0 mL) was added compound **6A** (2.05 g, 20.0 mmol), $NaBH(OAc)_3$ (6.36 g, 30.0 mmol) and HOAc (90.2 mg, 1.50 mmol). The mixture was stirred at 25 °C for 5 hrs. LCMS (product RT = 1.701 min) showed the starting

material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove DCM, then washed with H_2O 200 mL, and extracted with EtOAc 900 mL (300 mL x 3). The combined organic layers were washed with $NaHCO_3$ 200 mL and brine 200 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, DCM/MeOH =100/1 to 50/1). Compound 7 (4.60 g, 5.20 mmol, 51.9% yield) was obtained as a yellow oil.

General procedure for preparation of compound 9



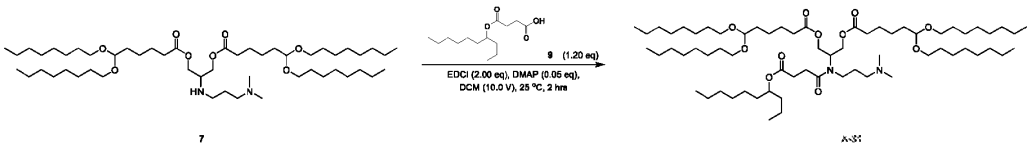
8 **9**

To a solution of compound **8** (10.0 g, 63.2 mmol) in THF (10.0 mL) was added DMAP (7.72 g, 63.2 mmol), Pyridine (9.99 g, 126 mmol) and tetrahydrofuran-2,5-dione (12.6 g, 126 mmol). The mixture was stirred at 50 °C for 8 hrs. The mixture was stirred at 70 °C for 6 hrs. LCMS (product RT = 1.717 min) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove THF, then the residue was washed with H_2O 150 mL, and extracted with MTBE 150 mL (50 mL x 3). The combined organic layers were washed with brine 50 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate=10/1 to 1/1). Compound **9** (10.0 g, 38.7 mmol, 61.3% yield) was obtained as a yellow oil.

^{1}H NMR: ET89868-6-P1A1 (400 MHz, $CDCl_3$)

δ = 12.17 (br s, 1H), 4.79 (s, 1H), 2.46 (s, 4H), 1.41 - 1.50 (m, 4H), 1.23 (br s, 10H), 0.85 (s, 6H)

General procedure for preparation of A-31



7 A-31

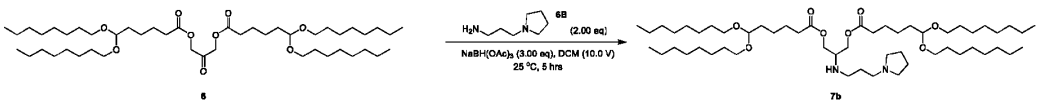
To a solution of compound **7** (4.50 g, 5.08 mmol) in DCM (45.0 mL) was added compound **9** (1.97 g, 7.62 mmol), EDCI (1.95 g, 10.2 mmol) and DMAP (31.1 mg, 254 μmol). The mixture was stirred at 25 °C for 2 hrs. LCMS (product RT = 2.545min) showed the starting material was consumed completely. The reaction mixture was diluted with DCM 30 mL, washed with brine 80 mL and extracted with DCM 150 mL (50 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, DCM/MeOH =100/1 to 10/1). **A-31** (601 mg, 534 μmol, 10.5% yield) was obtained as a yellow oil.

^{1}H NMR: ET89868-44-P1A1 (400 MHz, $CDCl_3$)

δ = 4.88 (br s, 1H), 4.44 (s, 3H), 4.10 - 4.37 (m, 3H), 3.86 (br s, 1H), 3.46 - 3.61 (m, 4H), 3.24 - 3.45 (m, 5H), 2.44 - 2.76 (m, 10H), 2.26 - 2.43 (m, 6H), 1.89 (br d, J = 6.9 Hz, 2H), 1.42 - 1.76 (m, 22H), 1.27 (br s, 54H), 0.88 (s, 18H)

LCMS:ET89868-44-P1B1 product: RT =3.439 min, $[M+H]^+$ = 1126.0, purity = 97.0%

General procedure for preparation of compound 7b

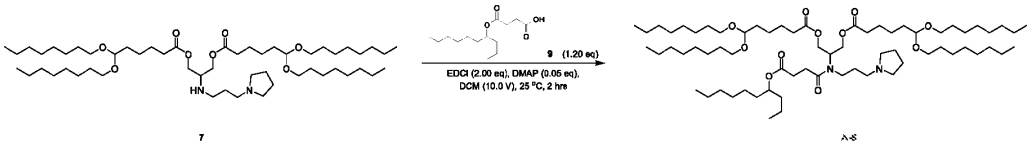


6 7b

To a solution of compound **6** (8 g, 10.0 mmol) in DCM (80.0 mL) was added compound **6B** (2.57 g, 20.0 mmol), $NaBH(OAc)_3$ (6.36 g, 30.0 mmol) and HOAc (90.2 mg, 1.50 mmol). The mixture was stirred at 25 °C for 5 hrs. LCMS (product RT = 1.773 min) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove DCM, then washed with H_2O 200 mL, and extracted with EtOAc 900 mL

(300 mL x 3). The combined organic layers were washed with $NaHCO_3$ 200 mL and brine 200 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, DCM/MeOH =100/1 to 50/1). Compound **7b** (4.00 g, 4.39 mmol, 43.84% yield) was obtained as a yellow oil.

General procedure for preparation of A-6



7 A-6

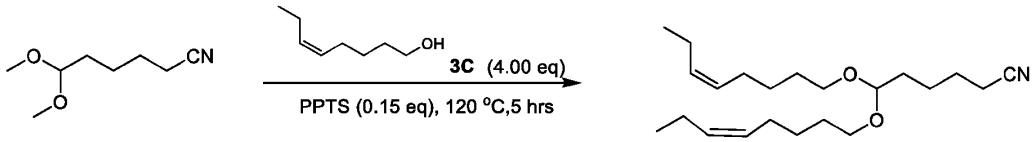
To a solution of compound **7b** (3.90 g, 4.28 mmol) in DCM (39.0 mL) was added compound **9** (1.66 g, 6.42 mmol), EDCI (1.64 g, 8.56 mmol) and DMAP (26.1 mg, 214μmol). The mixture was stirred at 25 °C for 1 hr. LCMS (product RT = 2.559 min) showed the starting material was consumed completely. The reaction mixture was diluted with DCM 30 mL, washed with brine 80 mL and extracted with DCM 150 mL (50 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, DCM/MeOH =100/1 to 10/1). **A-6** (604 mg, 524 μmol, 12.3% yield) was obtained as a yellow oil.

^{1}H NMR: ET89868-45-P1A1 (400 MHz, $CDCl_3$)

δ = 4.87 (br d, J = 1.9 Hz, 1H), 4.44 (s, 3H), 4.10 - 4.37 (m, 3H), 3.86 (br d, J = 5.1 Hz, 1H), 3.55 (br d, J = 9.3 Hz, 4H), 3.39 (br d, J = 9.3 Hz, 6H), 2.48 – 3.01 (m, 10H), 2.31 (br d, J = 7.3 Hz, 4H), 1.77 – 2.07 (m, 6H), 1.55 (br d, J = 7.1 Hz, 22H), 1.27 (br s, 54H), 0.88 (s, 18H)

LCMS:ET89868-45-P1B2 product: RT =3.529 min, $[M+H]^+$ = 1151.9, purity = 99.6%

General procedure for preparation of compound 4c



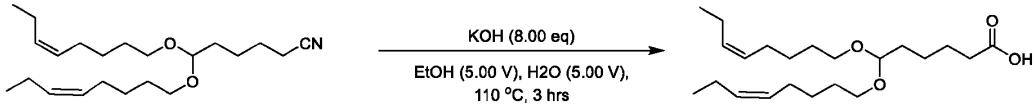
3 4c

To a solution of compound **3** (10.0 g, 63.61 mmol) in compound **3C** (32.6 g, 254 mmol,) was added PPTS (2.40 g, 9.54 mmol). The mixture was stirred at 120 °C for 5 hrs. TLC (Petroleum ether/Ethyl acetate = 3/1, product: R_f = 0.63, starting material: R_f = 0.28) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate=200/1 to 70/1). Compound **4c** (6.00 g, 17.2 mmol, 26.98% yield) was obtained as colorless oil.

^{1}H NMR: ET89868-21-P1A1 (400 MHz, $CDCl_3$)

δ = 5.23 - 5.51 (m, 4H), 4.46 (s, 1H), 3.57 (s, 2H), 3.42 (s, 2H), 2.35 (s, 2H), 2.05 (br d, J = 3.6 Hz, 8H), 1.57 (br d, J = 2.3 Hz, 10H), 1.42 (s, 4H), 0.96 (s, 6H)

General procedure for preparation of compound 5c



4c 5c

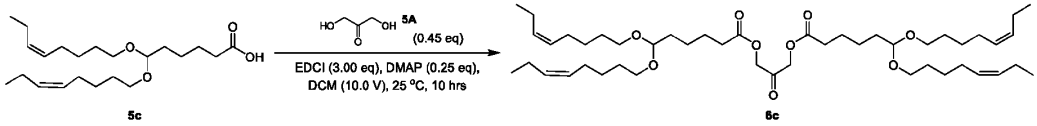
To a solution of compound **4c** (6.00 g, 17.2 mmol) in EtOH (30.0 mL) and H_2O (30.0 mL) was added KOH (7.70 g, 137 mmol). The mixture was stirred at 110 °C for 5 hrs. TLC (Petroleum ether/Ethyl acetate = 3/1, product: R_f = 0.45, starting material: R_f = 0.70) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove EtOH. The residue was diluted with H_2O 100 mL and acidified to PH = 5 using 1M HCl. Then, the mixture was extracted with EtOAc 450 mL (150 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced

pressure to give a residue. The crude product was used into the next step without further purification. Compound **5c** (6.10 g, crude) was obtained as a yellow oil.

^{1}H NMR: ET89868-34-P1B1 (400 MHz, DMSO-d_6)

δ = 5.22 – 5.49 (m, 4H), 4.40 (t, J = 5.6 Hz, 1H), 3.48 (td, J = 6.4, 9.4 Hz, 2H), 3.34 (td, J = 6.4, 9.4 Hz, 2H), 2.16 (t, J = 7.3 Hz, 2H), 1.99 (quin, J = 6.9 Hz, 8H), 1.43 - 1.52 (m, 8H), 1.24 - 1.39 (m, 6H), 0.91 (t, J = 7.6 Hz, 6H)

General procedure for preparation of compound 6c

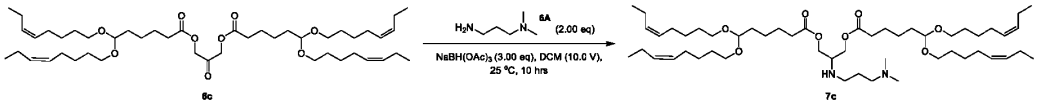


To a solution of compound **5c** (6.10 g, 16.6 mmol) in DCM (60.0 mL) was added compound **5A** (678 mg, 7.52 mmol), EDCI (4.33 g, 22.6 mmol) and DMAP (230 mg, 1.88 mmol). The mixture was stirred at 25 °C for 10 hrs. TLC (Petroleum ether/Ethyl acetate = 3/1, product: R_f = 0.61, starting material: R_f = 0.51) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove DCM. The residue was washed with H_2O 100 mL, and then extracted with Petroleum ether 450 mL (150 mL x 3). The combined organic layers were washed with brine 100 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. Compound **6c** (6.40 g, crude) was obtained as a yellow oil.

^{1}H NMR: ET89868-36-P1A2 (400 MHz, $CDCl_3$)

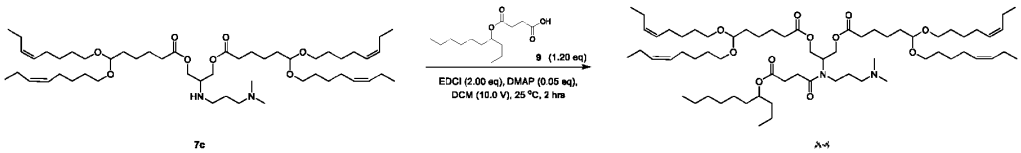
δ = 5.28 - 5.45 (m, 8H), 4.46 (t, J = 5.7 Hz, 2H), 3.57 (td, J = 6.5, 9.2 Hz, 4H), 3.41 (td, J = 6.6, 9.2 Hz, 4H), 2.44 (t, J = 7.6 Hz, 4H), 1.99 – 2.09 (m, 16H), 1.55 - 1.72 (m, 16H), 1.37 - 1.47 (m, 12H), 0.96 (t, J = 7.5 Hz, 12H)

General procedure for preparation of compound 7c



To a solution of compound **6c** (6.40 g, 8.09 mmol) in DCM (64.0 mL) was added compound **6A** (1.65 g, 16.2 mmol), $NaBH(OAc)_3$ (5.14 g, 24.3 mmol) and HOAc (72.9 mg, 1.21 mmol). The mixture was stirred at 25 °C for 5 hrs. LCMS (product RT = 1.701 min) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove DCM, then washed with H_2O 100 mL, and extracted with EtOAc 450 mL (150 mL x 3). The combined organic layers were washed with $NaHCO_3$ 100 mL and brine 100 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, DCM/MeOH =100/1 to 50/1). Compound **7c** (3.80 g, 4.33 mmol, 53.5% yield) was obtained as a yellow oil.

General procedure for preparation of A-4



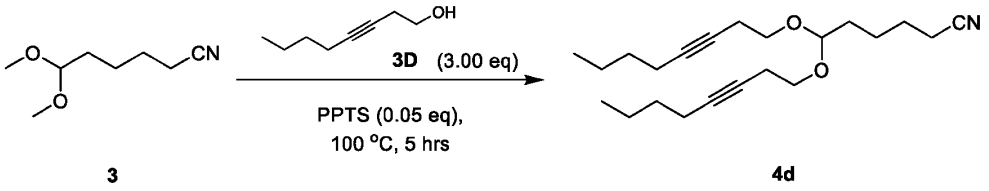
To a solution of compound **7c** (3.70 g, 4.22 mmol) in DCM (37.0 mL) was added compound **9** (1.63 g, 6.33 mmol), EDCI (1.62 g, 8.43 mmol) and DMAP (25.8 mg, 211μmol). The mixture was stirred at 25 °C for 2 hrs. TLC (DCM/MeOH = 5/1, product: R_f = 0.59, starting material: R_f = 0.56) showed the starting material was consumed completely. The reaction mixture was diluted with DCM 30 mL, washed with brine 80 mL and extracted with DCM 150 mL (50 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, DCM/MeOH =100/1 to 10/1). **A-4** (615 mg, 550 μmol, 13.1% yield) was obtained as a yellow oil.

^{1}H NMR: ET89868-45-P1A1 (400 MHz, $CDCl_3$)

δ = 5.20 - 5.42 (m, 8H), 4.77 - 4.85 (m, 1H), 4.32 - 4.41 (m, 3H), 4.03 - 4.29 (m, 3H), 3.67 - 3.92 (m, 1H), 3.44 - 3.53 (m, 4H), 3.16 - 3.39 (m, 6H), 2.28 - 2.59 (m, 10H), 2.21 - 2.31 (m, 6H), 1.92 – 2.02 (m, 14H), 1.80 (br d, J = 6.9 Hz, 2H), 1.46 - 1.61 (m, 18H), 1.29 - 1.38 (m, 12H), 1.14 - 1.26 (m, 14H), 0.80 - 0.91 (m, 18H)

LCMS:ET89868-46-P1B2 product: RT =3.131 min, $[M+H]^+$ = 1117.9, purity = 96.1%

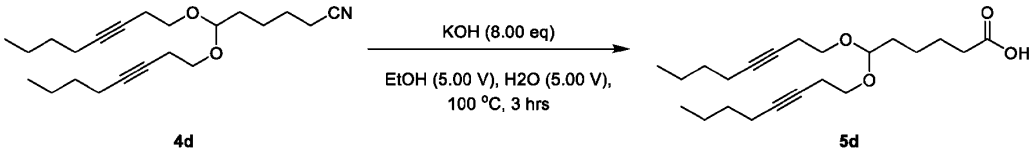
General procedure for preparation of compound



3 **4d**

To a solution of compound **3** (10.0 g, 63.6 mmol) in compound **3D** (24.1 g, 191 mmol) was added PPTS (2.40 g, 9.54 mmol). The mixture was stirred at 100 °C for 5 hrs. TLC (Petroleum ether/Ethyl acetate = 3/1, product: R_f = 0.63, starting material: R_f = 0.28) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to give a residue. The crude product was distilled to remove excessive cpd.3D, and the remained crude product was used into the next step. Compound **4d** (16.9 g, crude) was obtained as colorless oil.

General procedure for preparation of compound 5d



4d **5d**

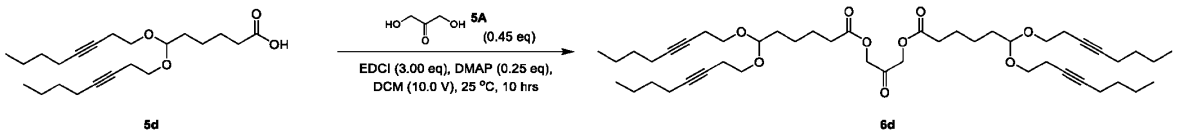
To a solution of compound **4d** (15.9 g, 46.0 mmol) in EtOH (80.0 mL) and H_2O (80.0 mL) was added KOH (12.9 g, 230 mmol). The mixture was stirred at 100 °C for 5 hrs. TLC (Petroleum ether/Ethyl acetate = 3/1, product: R_f = 0.34, starting material: R_f = 0.52) showed the starting

material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove EtOH. The residue was diluted with H_2O 150 mL and acidified to PH = 5 using 1M HCl. Then, the mixture was extracted with EtOAc 600 mL (200 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate=100/1 to 10/1). Compound **5d** (6.00 g, 16.5 mmol, 35.8% yield) was obtained as a yellow oil.

^{1}H NMR: ET89868-52-P1A1 (400 MHz, DMSO-d_6)

δ = 11.96 (br s, 1H), 4.52 (t, J = 5.7 Hz, 1H), 3.56 (td, J = 6.8, 9.3 Hz, 2H), 3.45 (td, J = 6.8, 9.3 Hz, 2H), 2.31 - 2.37 (m, 4H), 2.18 (t, J = 7.4 Hz, 2H), 2.12 (ddd, J = 2.3, 4.4, 6.8 Hz, 4H), 1.45 - 1.55 (m, 4H), 1.26 - 1.43 (m, 10H), 0.82 - 0.90 (m, 6H)

General procedure for preparation of compound 6d



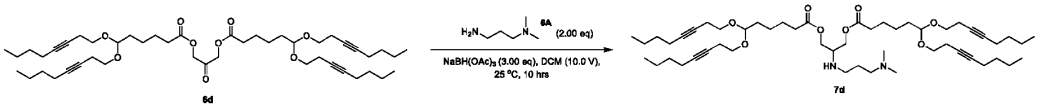
5d 6d

To a solution of compound **5d** (3.00 g, 8.23 mmol) in DCM (30.0 mL) was added compound **5A** (337 mg, 3.74 mmol), EDCI (2.15 g, 11.2 mmol) and DMAP (45.7 mg, 374 μmol). The mixture was stirred at 25 °C for 5 hrs. TLC (Petroleum ether/Ethyl acetate = 3/1, product: R_f = 0.51, starting material: R_f = 0.36) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove DCM. The residue was washed with H_2O 20 mL, and then extracted with Petroleum ether 30 mL (30 mL x 3). The combined organic layers were washed with brine 30 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. Compound **6d** (3.00 g, crude) was obtained as a yellow oil.

^{1}H NMR: ET89868-58-P1A1 (400 MHz, $CDCl_3$)

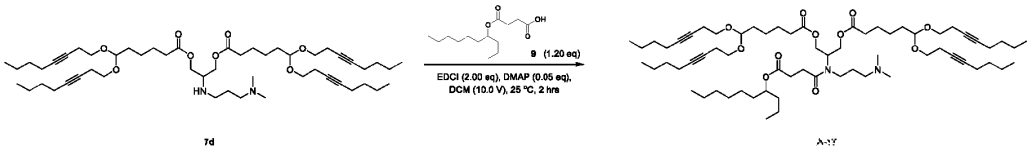
δ = 4.74 (s, 4H), 4.56 (t, J = 5.8 Hz, 2H), 3.66 (td, J = 7.1, 9.1 Hz, 4H), 3.54 (td, J = 7.2, 9.1 Hz, 4H), 2.38 - 2.46 (m, 12H), 2.14 (tt, J = 2.3, 6.9 Hz, 8H), 1.61 - 1.74 (m, 8H), 1.35 - 1.50 (m, 20H), 0.86 - 0.95 (m, 12H)

General procedure for preparation of compound 7d



To a solution of compound **6d** (3.00 g, 3.83 mmol) in DCM (30.0 mL) was added compound **6A** (783 mg, 7.66 mmol), $NaBH(OAc)_3$ (2.44 g, 11.5 mmol) and HOAc (34.5 mg, 575 μmol). The mixture was stirred at 25 °C for 5 hrs. TLC (DCM/MeOH = 5/1, product: R_f = 0.52, starting material: R_f = 0.82) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove DCM, then washed with H_2O 30 mL, and extracted with EtOAc 150 mL (50 mL x 3). The combined organic layers were washed with $NaHCO_3$ 50 mL and brine 50 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, DCM/MeOH =100/1 to 5/1). Compound **7c** (2.60 g, 2.99 mmol, 78.1% yield) was obtained as a yellow oil.

General procedure for preparation of A-17



To a solution of compound **7d** (2.60 g, 2.99 mmol) in DCM (25.0 mL) was added compound **9** (927 mg, 3.59 mmol), EDCI (1.15 g, 5.98 mmol) and DMAP (18.3 mg, 150μmol). The mixture was stirred at 25 °C for 2 hrs. TLC (DCM/MeOH = 5/1, product: R_f = 0.60, starting material: R_f = 0.45) showed the starting material was consumed completely. The reaction mixture was diluted with DCM 30 mL, washed with brine 80 mL and extracted with DCM 150 mL (50 mL x

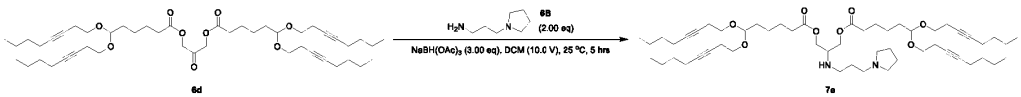
3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, DCM/MeOH =100/1 to 10/1). **A-17** (608 mg, 548 μmol, 18.3% yield) was obtained as a yellow oil.

^{1}H NMR: ET89868-61-P1A4 (400 MHz, $CDCl_3$)

δ = 4.85 (br d, J = 5.9 Hz, 1H), 4.55 (t, J = 5.6 Hz, 2H), 4.38 - 4.50 (m, 1H), 4.25 - 4.36 (m, 2H), 4.15 - 4.24 (m, 1H), 3.61 - 3.70 (m, 4H), 3.51 - 3.60 (m, 4H), 3.33 - 3.49 (m, 2H), 2.91 (br s, 1H), 2.54 - 2.73 (m, 10H), 2.42 (tt, J = 2.2, 7.0 Hz, 8H), 2.33 (td, J = 7.7, 15.7 Hz, 4H), 2.14 (tt, J = 2.3, 6.8 Hz, 8H), 1.96 – 2.10 (m, 2H), 1.58 - 1.68 (m, 8H), 1.32 - 1.56 (m, 26H), 1.26 (br s, 10H), 0.85 - 0.94 (m, 18H)

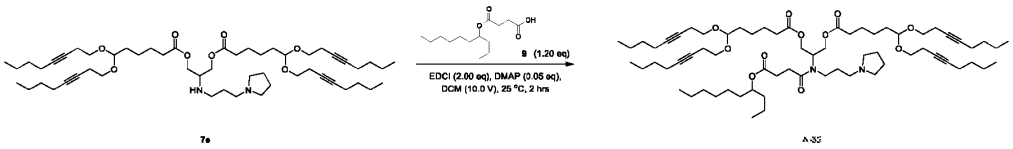
LCMS:ET89868-61-P1A1 product: RT =2.710 min, $[M+H]^+$ = 1109.9, purity = 99.9%

General procedure for preparation of compound 7e



To a solution of compound **6d** (600 mg, 766 μmol) in DCM (6.00 mL) was added compound **6B** (196 mg, 1.53 mmol), $NaBH(OAc)_3$ (487 mg, 2.30 mmol) and HOAc (6.90 mg, 115 μmol). The mixture was stirred at 25 °C for 5 hrs. TLC (DCM/MeOH = 5/1, product: R_f = 0.34, starting material: R_f = 0.80) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove DCM, then washed with H_2O 10 mL, and extracted with EtOAc 60 mL (20 mL x 3). The combined organic layers were washed with $NaHCO_3$ 30 mL and brine 30 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, DCM/MeOH =100/1 to 10/1). Compound **7e** (380 mg, 424 μmol, 55.4% yield) was obtained as a yellow oil.

General procedure for preparation of A-32



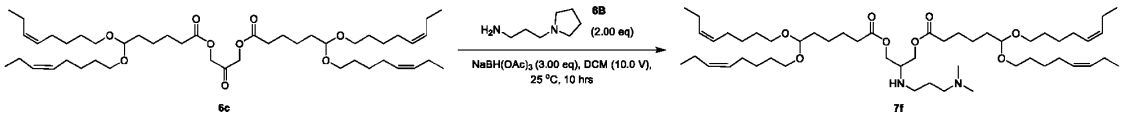
To a solution of compound **7e** (380 mg, 424 μmol) in DCM (4.00 mL) was added compound **9** (132 mg, 509 μmol), EDCI (163 mg, 849 μmol) and DMAP (1.59 mg, 21.2 μmol). The mixture was stirred at 25 °C for 2 hrs. TLC (DCM/MeOH = 10/1, product: R_f = 0.53, starting material: R_f = 0.38) showed the starting material was consumed completely. The reaction mixture was diluted with DCM 10 mL, washed with brine 20 mL and extracted with DCM 90 mL (30 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, DCM/MeOH =100/1 to 10/1). **A-32** (137 mg, 118 μmol, 27.9% yield) was obtained as a yellow oil.

^{1}H NMR: ET89868-124-P1A1 (400 MHz, $CDCl_3$)

δ = 4.87 (br s, 1H), 4.56 (br s, 2H), 4.39 - 4.50 (m, 1H), 4.33 (br s, 2H), 4.22 (br s, 1H), 3.65 (s, 4H), 3.56 (s, 4H), 3.41 (br s, 2H), 3.02 (br s, 1H), 2.60 (br s, 6H), 2.42 (br s, 8H), 2.31 (br s, 4H), 2.15 (br s, 14H), 1.62 (br s, 8H), 1.40 (br s, 26H), 1.27 (br s, 12H), 0.91 (s, 18H)

LCMS:ET89868-124-P1B1 product: RT =2.698 min, $[M+H]^+$ = 1135.8, purity = 98.2%

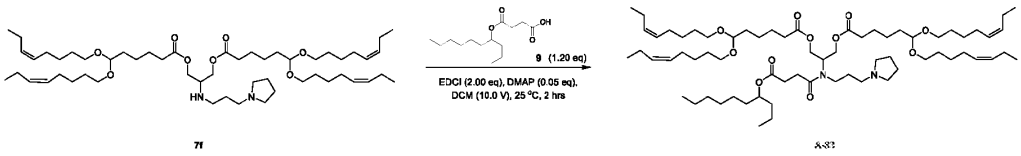
General procedure for preparation of compound 7f



To a solution of compound **6c** (630 g, 796 μmol) in DCM (6.00 mL) was added compound **6B** (204 mg, 1.59 mmol), $NaBH(OAc)_3$ (506 mg, 2.39 mmol) and HOAc (7.17 mg, 119 μmol). The mixture was stirred at 25 °C for 5 hrs. TLC (DCM/MeOH = 5/1, product: R_f = 0.55, starting material: R_f = 0.94) showed the starting material was consumed completely. The reaction

mixture was concentrated under reduced pressure to remove DCM, then washed with H_2O 10 mL, and extracted with EtOAc 90 mL (30 mL x 3). The combined organic layers were washed with $NaHCO_3$ 50 mL and brine 50 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, DCM/MeOH =100/1 to 5/1). Compound **7c** (300 mg, 332 μmol, 41.7% yield) was obtained as a yellow oil.

General procedure for preparation of A-33



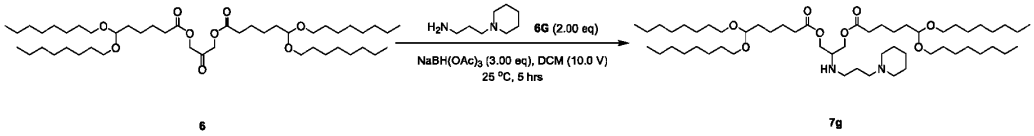
To a solution of compound **7f** (300 mg, 332 μmol) in DCM (3.00 mL) was added compound **9** (103 mg, 399 μmol), EDCI (127 mg, 664 μmol) and DMAP (2.03 mg, 16.6 μmol). The mixture was stirred at 25 °C for 2 hrs. TLC (DCM/MeOH = 10/1, product: R_f = 0.47, starting material: R_f = 0.38) showed the starting material was consumed completely. The reaction mixture was diluted with DCM 10 mL, washed with brine 10 mL and extracted with DCM 90 mL (30 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, DCM/MeOH =100/1 to 10/1). **A-33** (138 mg, 119 μmol, 35.8% yield) was obtained as a yellow oil.

^{1}H NMR: ET89868-116-P1A1 (400 MHz, $CDCl_3$)

δ = 5.35 (s, 8H), 4.88 (br s, 1H), 4.45 (br s, 7H), 3.55 (br s, 4H), 3.42 (br s, 6H), 2.60 (br d, J = 9.5 Hz, 6H), 2.31 (br s, 4H), 2.04 (br d, J = 2.3 Hz, 20H), 1.59 (br d, J = 6.9 Hz, 22H), 1.41 (br s, 12H), 1.26 (br s, 14H), 0.96 (s, 18H)

LCMS:ET89868-116-P1B1 product: RT =3.136 min, $[M+H]^+$ = 1143.9, purity = 98.6%

General procedure for preparation of compound 7g

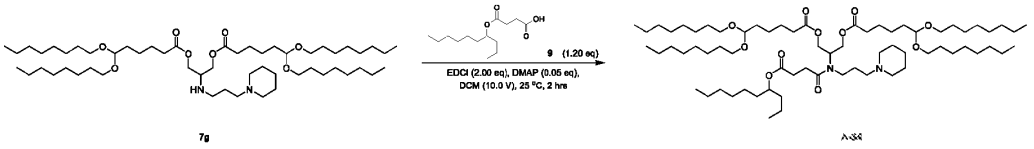


To a solution of compound **6** (0.70 g, 875 μmol) and compound **6G** (249 mg, 1.75 mmol) in DCM (7.00 mL) was added $NaBH(OAc)_3$ (556 mg, 2.63 mmol). The mixture was stirred at 25 °C for 2 hrs. LCMS (ET89944-90-P1A1, RT=4.186 min) show compound **6** was consumed and desired compound was detected. First, the reaction mixture was concentrated under reduced pressure to give a residue. Second, the residue was diluted with H_2O (50.0 mL) and extracted with EtOAc (50.0 mL x 3). The combined organic layers were washed with brine (50.0 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=10/1 to 0/1). Compound **7g** (0.25 g, 270 μmol, 30.8% yield) was obtained as a yellow oil.

^{1}H NMR: ET89944-90-P1A1 (400 MHz, $CDCl_3$)

δ = 4.43 - 4.50 (m, 2 H), 4.10 (br d, J=5.13 Hz, 2 H), 3.51 - 3.61 (m, 5 H), 3.34 - 3.47 (m, 5 H), 3.12 - 3.17 (m, 1 H), 3.00 - 3.08, (m, 1 H), 2.83 - 2.91 (m, 1 H), 2.70 (br s, 2 H), 2.28 - 2.40 (m, 4 H), 2.02 - 2.09 (m, 2 H), 1.83 (br s, 2 H), 1.51 - 1.68 (m, 16 H), 1.21 - 1.47 (m, 47 H), 0.85 - 0.93 (m, 12 H)

General procedure for preparation of A-34



To a solution of compound **7g** (200 mg, 216 μmol) in DCM (2.00 mL) was added compound **9** (83.7 mg, 323 μmol), EDCI (216 mg, 649 μmol) and DMAP (1.32 mg, 10.8 μmol). The mixture was stirred at 25 °C for 2 hrs. TLC (DCM/MeOH = 10/1, product: R_f = 0.56, starting material: R_f = 0.42) showed the starting material was consumed completely. The reaction mixture was

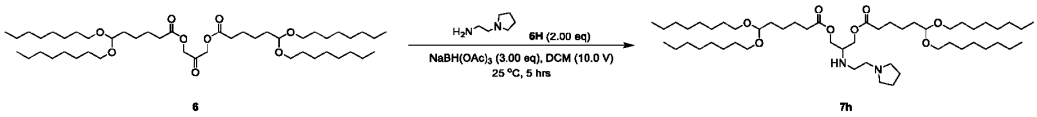
diluted with DCM 10 mL, washed with brine 10 mL and extracted with DCM 90 mL (30 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (TFA condition). **A-34** (80.0 mg, 61.6 μmol, 8.14% yield) was obtained as a yellow oil.

^{1}H NMR: ET89868-134-P1A1 (400 MHz, $CDCl_3$)

δ = 4.81 - 4.92 (m, 1H), 4.45 (s, 3H), 4.30 - 4.38 (m, 1H), 4.11 - 4.27 (m, 3H), 3.52 - 3.67 (m, 6H), 3.41 (s, 4H), 2.96 - 3.08 (m, 2H), 2.46 - 2.68 (m, 7H), 2.27 - 2.41 (m, 5H), 1.78 - 2.07 (m, 7H), 1.58 (br s, 60H), 1.28 (br s, 56H), 0.89 (s, 18H)

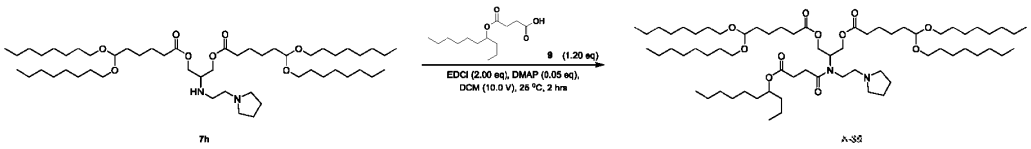
LCMS:ET89868-134-P1B4 product: RT =3.473 min, $[M+H]^+$ = 1165.9, purity = 98.5%

General procedure for preparation of compound 7h



To a solution of compound **6** (0.70 g, 875 μmol) and compound **6H** (200 mg, 1.75 mmol) in DCM (7.00 mL) was added $NaBH(OAc)_3$ (556 mg, 2.63 mmol). The mixture was stirred at 25 °C for 1 hr. LCMS (ET89944-89-P1A1, RT=4.523 min) show compound **6** was consumed and desired compound was detected. First, the reaction mixture was concentrated under reduced pressure to give a residue. Second, the residue was diluted with H_2O (50.0 mL) and extracted with EtOAc (50.0 mL x 3). The combined organic layers were washed with brine (50.0 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. Compound **7h** (700 mg, crude) was obtained as a yellow oil.

General procedure for preparation of A-35



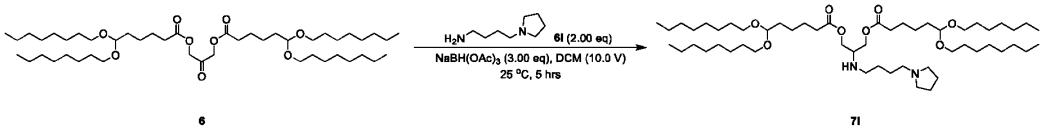
To a solution of compound **7h** (200 mg, 223 μmol) in DCM (2.00 mL) was added compound **9** (86.4 mg, 334 μmol), EDCI (128 mg, 668 μmol) and DMAP (1.36 mg, 11.1 μmol). The mixture was stirred at 25 °C for 2 hrs. TLC (DCM/MeOH = 10/1, product: R_f = 0.52, starting material: R_f = 0.32) showed the starting material was consumed completely. The reaction mixture was diluted with DCM 10 mL, washed with brine 10 mL and extracted with DCM 90 mL (30 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, DCM/MeOH = 100/1 to 10/1). **A-35** (90.0 mg, 71.2 μmol, 31.9% yield) was obtained as a yellow oil.

^{1}H NMR: ET89868-127-P1A1 (400 MHz, $CDCl_3$)

δ = 4.89 (br s, 1H), 4.46 (br d, J = 5.3 Hz, 5H), 4.13 (s, 2H), 3.56 (br d, J = 9.1 Hz, 4H), 3.41 (s, 6H), 2.61 (s, 8H), 2.31 (br s, 4H), 1.80 (br s, 4H), 1.56 (br s, 28H), 1.25 - 1.36 (m, 48H), 0.89 (s, 18H)

LCMS:ET89868-127-P1B4 product: RT =3.455 min, $[M+H]^+$ = 1137.9, purity = 90.6%

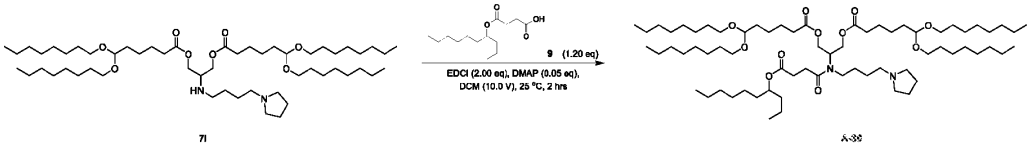
General procedure for preparation of compound 7i



To a solution of compound **6** (0.70 g, 875 μmol) and compound **6I** (249 mg, 1.75 mmol) in DCM (7.00 mL) was added $NaBH(OAc)_3$ (556 mg, 2.63 mmol). The mixture was stirred at 25 °C for

1hr. LCMS (ET89944-91-P1A1, RT=4.113 min) show compound **6** was consumed and desired compound was detected. First, the reaction mixture was concentrated under reduced pressure to give a residue. Second, the residue was diluted with H_2O (50.0 mL) and extracted with EtOAc (50.0 mL x 3). The combined organic layers were washed with brine (50.0 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. Compound **7i** (700 mg, crude) was obtained as a yellow oil.

General procedure for preparation of A-36



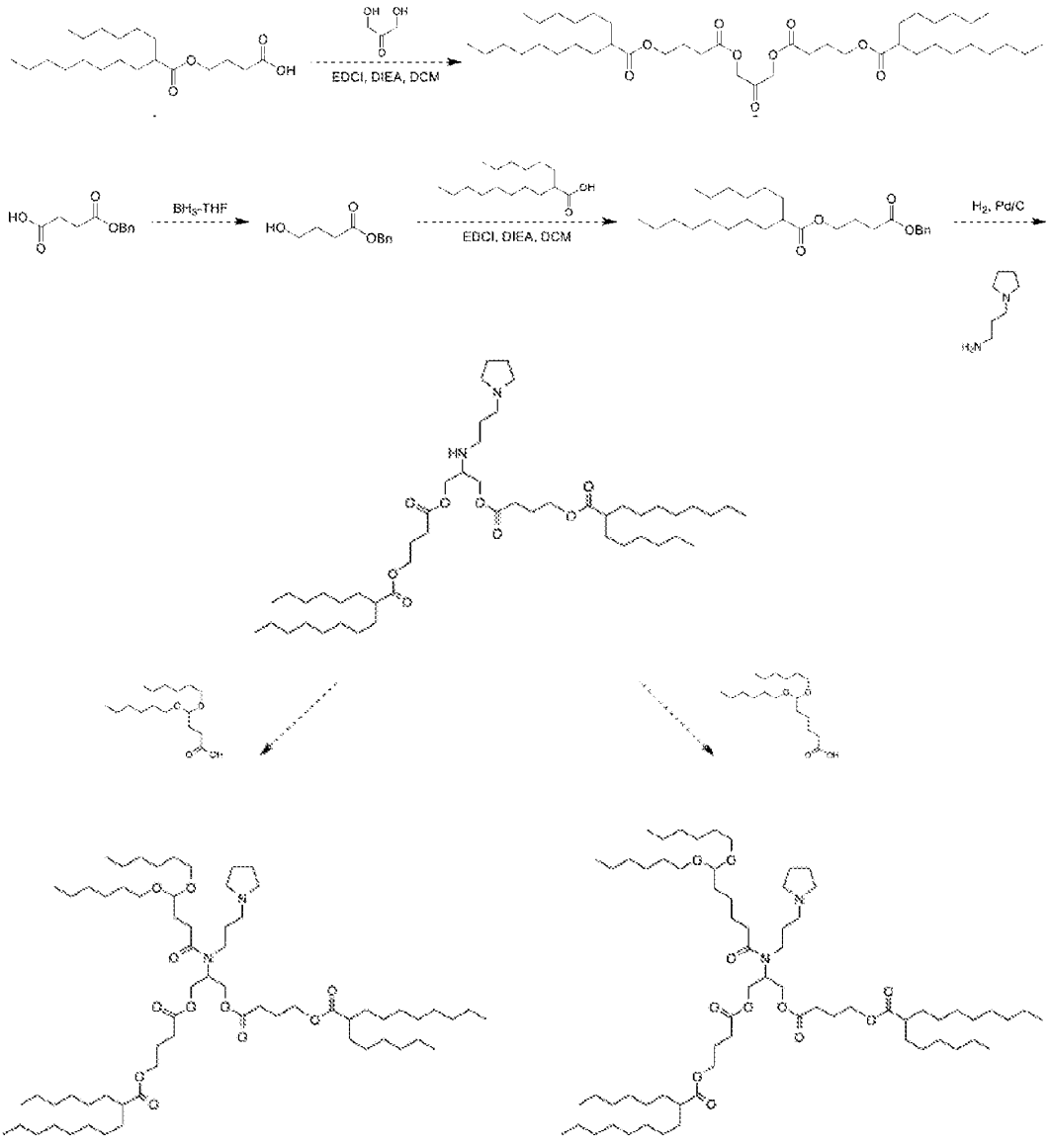
To a solution of compound **7i** (200 mg, 216.11 μmo) in DCM (2.00 mL) was added compound **9** (83.8 mg, 324 μmol), EDCI (124 mg, 648 μmol) and DMAP (1.32 mg, 10.8 μmol). The mixture was stirred at 25 °C for 2 hrs. TLC (DCM/MeOH = 10/1, product: R_f = 0.55, starting material: R_f = 0.46) showed the starting material was consumed completely. The reaction mixture was diluted with DCM 10 mL, washed with brine 10 mL and extracted with DCM 90 mL (30 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, DCM/MeOH =100/1 to 10/1). **A-36** (136 mg, 113 μmol, 52.4% yield) was obtained as a yellow oil.

^{1}H NMR: ET89868-135-P1A1 (400 MHz, $CDCl_3$)

δ = 4.79 - 4.99 (m, 2H), 4.45 (s, 3H), 4.27 - 4.37 (m, 1H), 4.06 - 4.26 (m, 2H), 3.54 (s, 4H), 3.41 (s, 4H), 3.18 - 3.33 (m, 2H), 2.62 (s, 8H), 2.31 (br s, 4H), 1.82 (br s, 2H), 1.45 - 1.72 (m, 24H), 1.28 (br s, 58H), 0.89 (s, 18H).

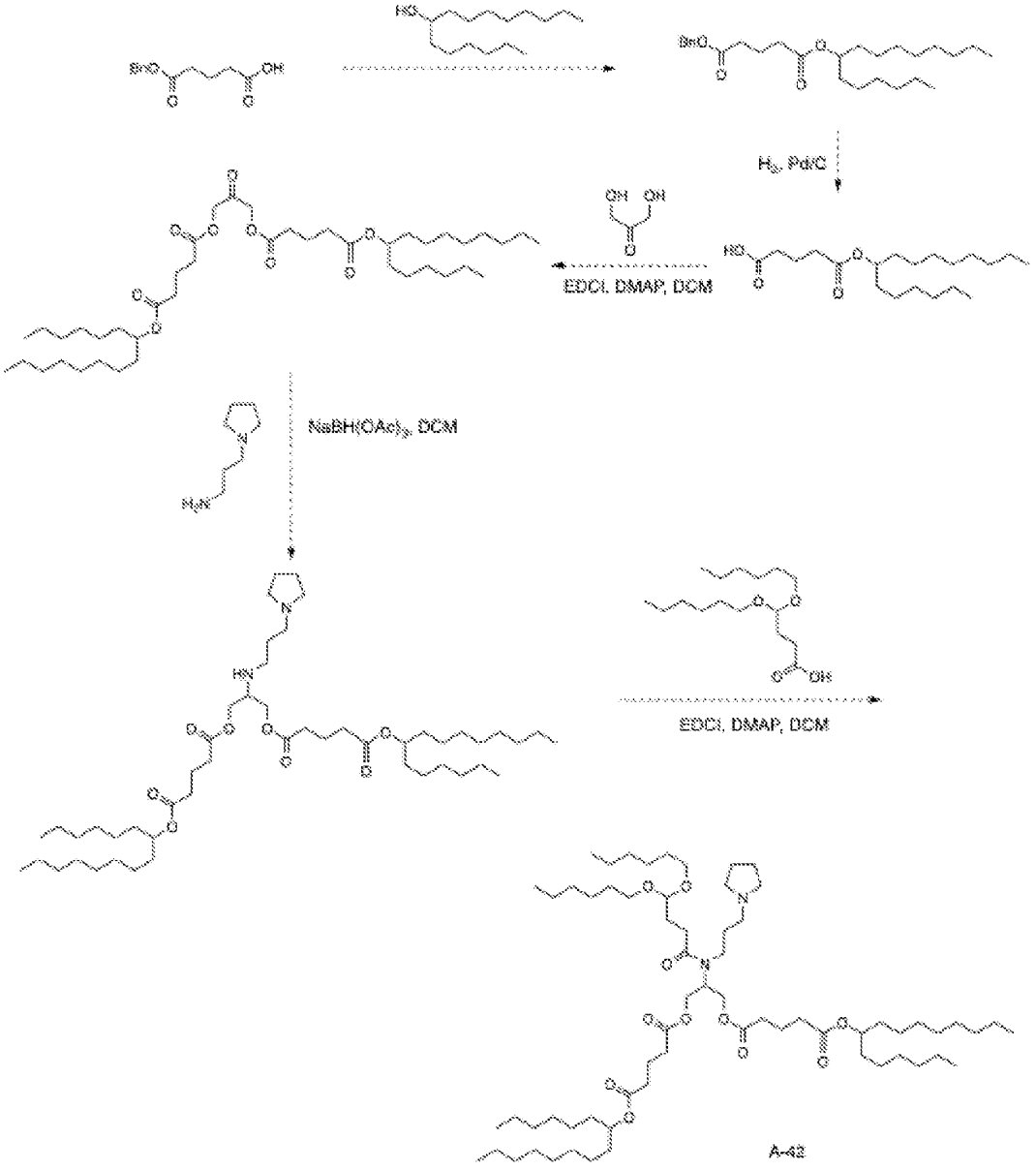
LCMS:ET89868-135-P1B2 product: RT =3.429min, $[M+H]^+$ = 1166.0, purity = 97.1%

General procedure for preparation of A-40 and A-41

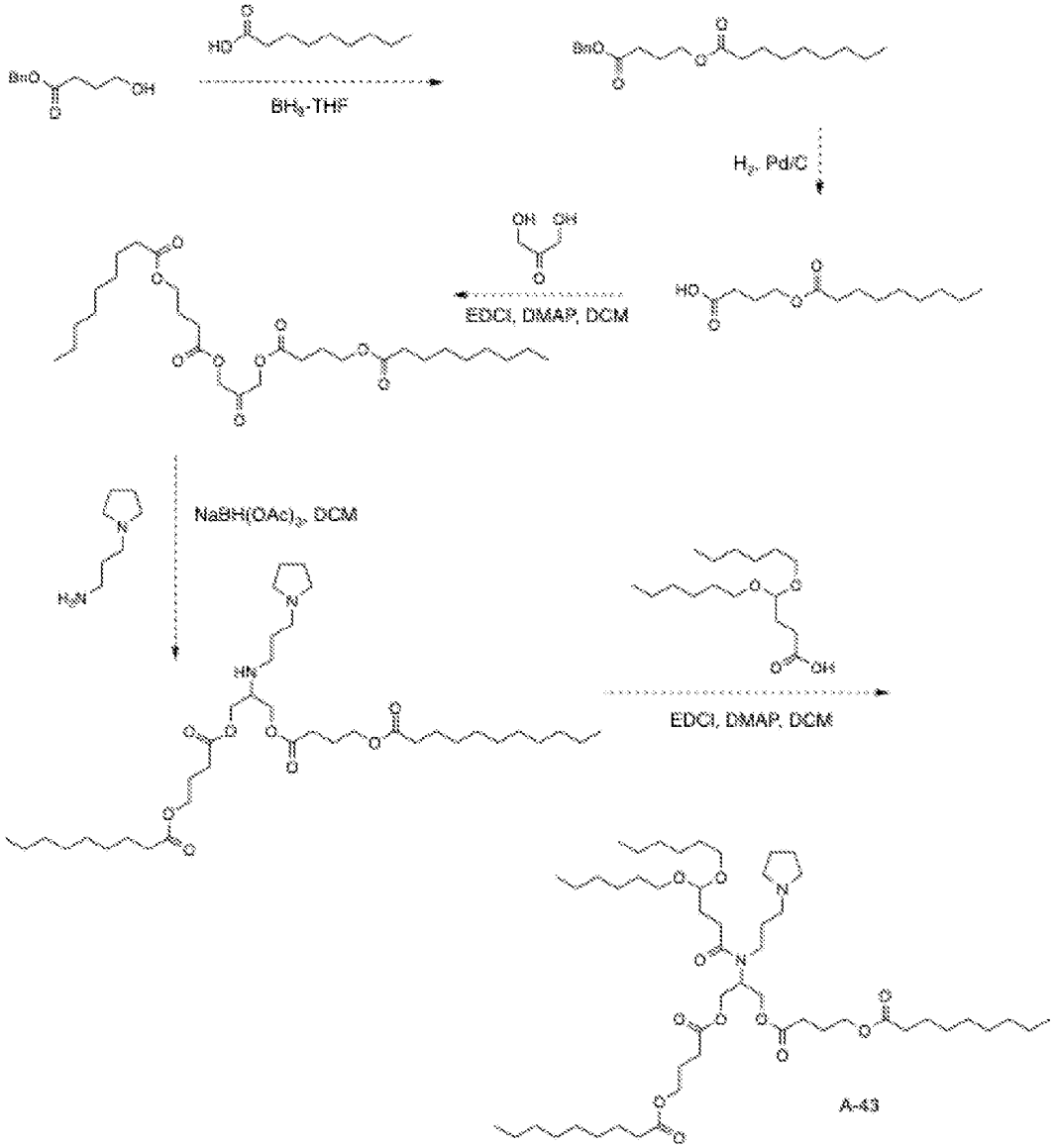


A-40 A-41

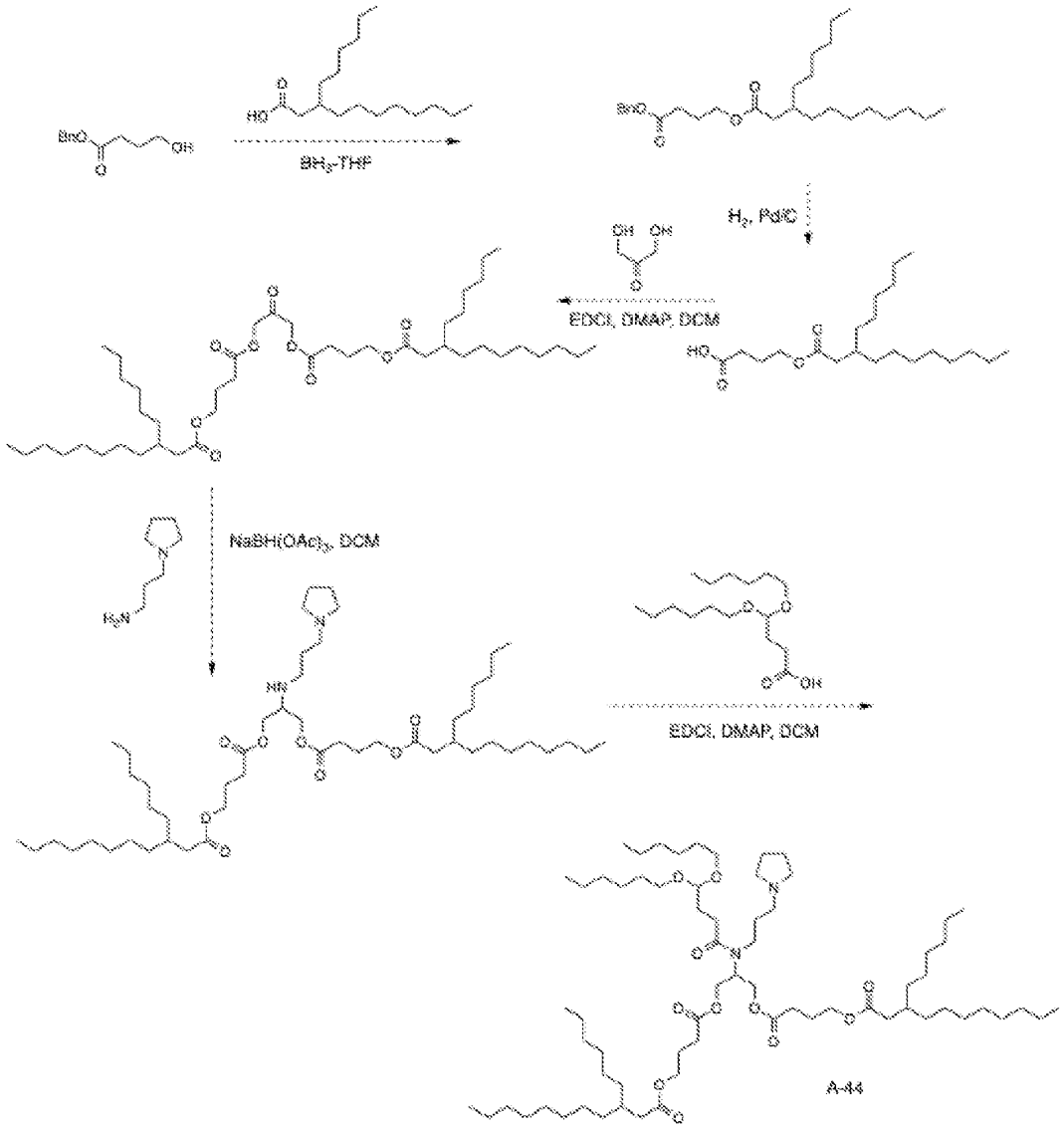
General procedure for preparation of A-42



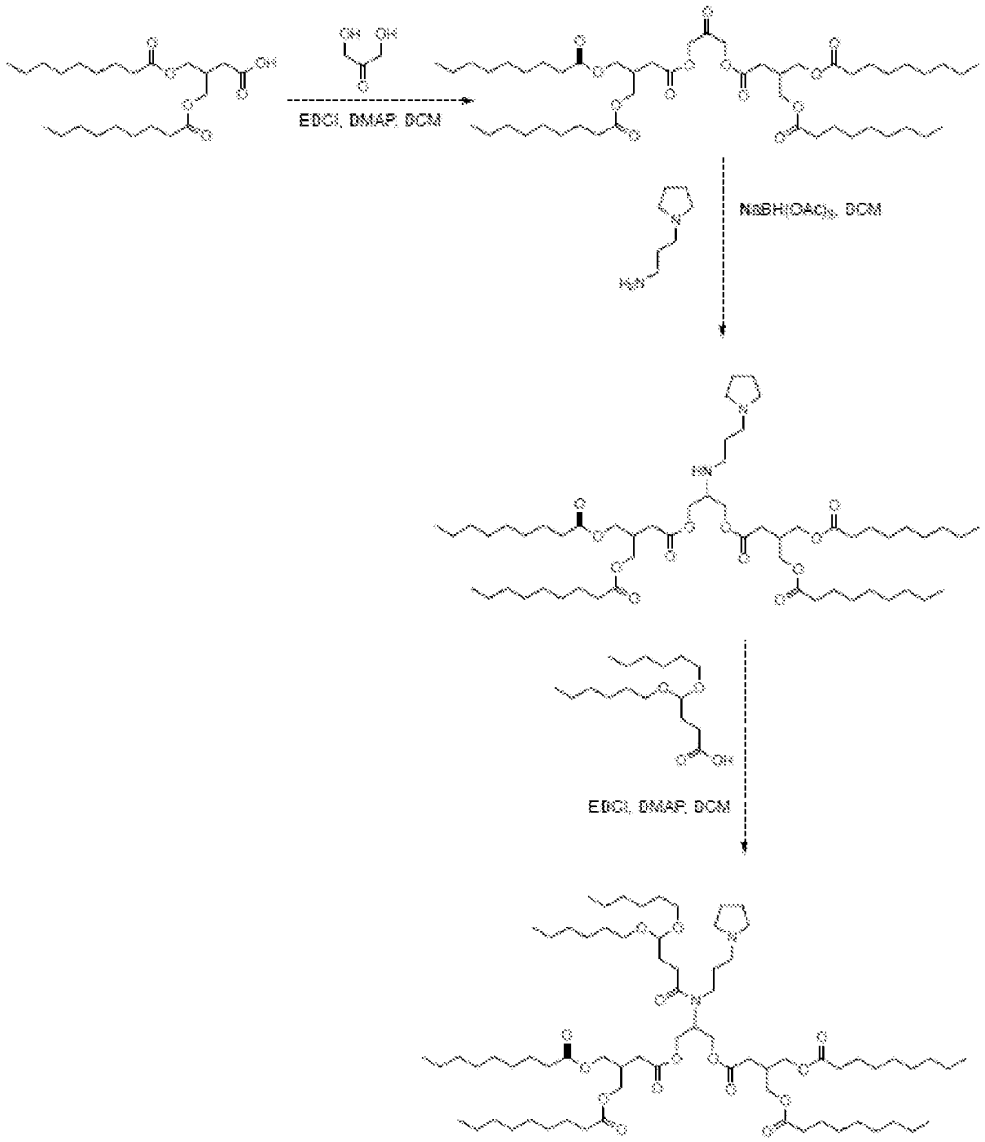
General procedure for preparation of A-43



General procedure for preparation of A-44



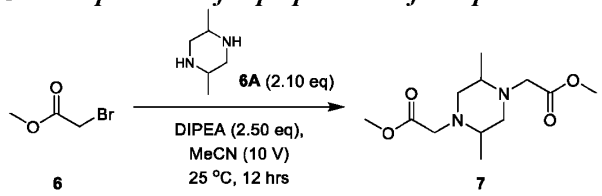
General procedure for preparation of A-45



A45

Synthesis of B-9

General procedure for preparation of compound 7

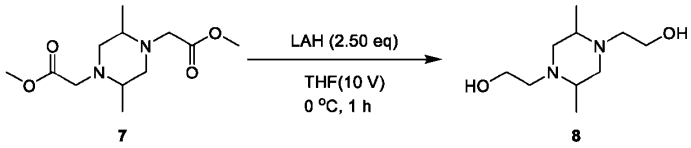


To a solution of compound **6** (0.80 g, 7.01 mmol) in MeCN (8.00 mL) was added TEA (2.26 g, 17.5 mmol) and compound **6A** (2.25 g, 14.7 mmol). The mixture was stirred at 25 °C for 12 hrs. LCMS (product RT = 0.101 min) showed the starting material was consumed completely. The mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1) to give compound **7** (1.60 g, 6.19 mmol, 88.4% yield) as colorless oil.

^{1}H NMR: ET89912-8-P1B (400 MHz, $CDCl_3$)

δ 3.70 (s, 6H), 3.43 - 3.47 (m, 2H), 3.21 - 3.25 (m, 2H), 2.76 - 2.79 (m, 2H), 2.66 - 2.69 (m, 2 H), 2.31 - 2.37 (m, 2H), 1.02 - 1.07 (m, 6H).

General procedure for preparation of compound 8

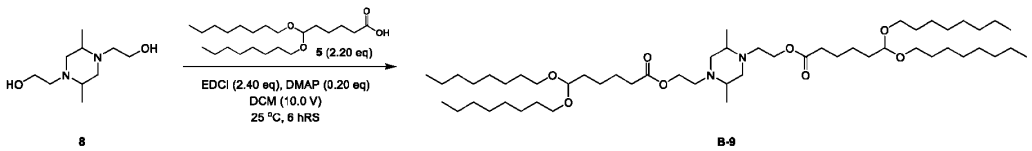


To a solution of compound **7** (1.40 g, 5.42 mmol) in THF (14.0 mL) was added LAH (0.51 g, 13.5 mmol, 5.42 mL, 2.50 M) at 0 °C. The mixture was stirred at 0 °C for 1 hr. LCMS (product RT = 0.093 min) showed the starting material was consumed completely. The reaction mixture was quenched by water (0.50 mL). Then the mixture was filtered and the filtrate was concentrated under reduced pressure to give compound **8** (1.00 g, crude) as off-white solid.

^{1}H NMR: ET89912-8-P1B (400 MHz, $CDCl_3$)

δ 3.62 - 3.67 (m, 4H), 2.86 - 2.94 (m, 4H), 2.42 - 2.45 (m, 2H), 2.32 - 2.35 (m, 2H), 2.08 - 2.11 (m, 2 H), 1.06 - 1.08 (m, 6H).

General procedure for preparation of compound B-9



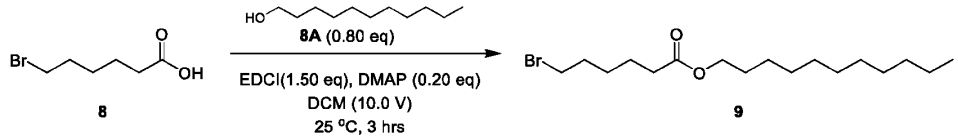
To a solution of compound **8** (0.90 g, 4.45 mmol) in DCM (9.00 mL) was added compound **5** (3.65 g, 9.79 mmol) and EDCI (2.05 g, 10.7 mmol) and DMAP (0.11 g, 0.89 mmol). The mixture was stirred at 25 °C for 6 hrs. LCMS (product RT = 4.65 min) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove DCM. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1) to give **B-9** (0.54 g, 0.59 mmol, 13.3% yield) as light yellow oil.

^{1}H NMR: ET89912-18-P1B2 (400 MHz, $CDCl_3$)

δ 4.45 (t, J = 5.7 Hz, 2H), 4.11 - 4.21 (m, 4H), 3.55 (td, J = 6.7, 9.2 Hz, 4H), 3.40 (td, J = 6.7, 9.2 Hz, 4H), 2.97 (td, J = 6.6, 13.6 Hz, 2H), 2.79 (br dd, J = 2.3, 11.1 Hz, 2H), 2.41 - 2.60 (m, 4H), 2.31 (t, J = 7.6 Hz, 4H), 2.14 (br t, J = 10.6 Hz, 2H), 1.50 - 1.68 (m, 16H), 1.21 - 1.41 (m, 44H), 0.99 - 1.12 (m, 6H), 0.84 - 0.94 (m, 12H).

Synthesis of B-10

General procedure for preparation of compound 9

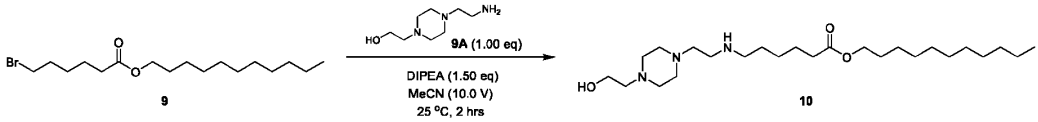


To a solution of compound **8A** (10.0 g, 58.0 mmol) in DCM (100 mL) was added compound **8** (13.6 g, 69.6 mmol), DMAP (1.42 g, 11.6 mmol) and EDCI (16.7 g, 87.1 mmol). The mixture was stirred at 25 °C for 3 hrs. LCMS (product RT = 2.42 min) showed the starting material was consumed completely. The reaction mixture was added H_2O (100 mL) and extracted with DCM (100 mL x 3). The combined organic layers were washed with bine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give compound **9** (20.0 g, crude) as colorless oil.

^{1}H NMR: ET89912-8-P1B (400 MHz, $CDCl_3$)

δ 4.04 - 4.08 (m, 2H), 3.39 - 3.43 (m, 2H), 2.30 - 2.34 (m, 2H), 1.68 - 1.88 (m, 2 H), 1.60 - 1.66 (m, 4H), 1.40 - 1.48 (m, 2H), 1.26 - 1.31 (m, 16H), 0.86 - 0.90 (m, 3H).

General procedure for preparation of compound 10

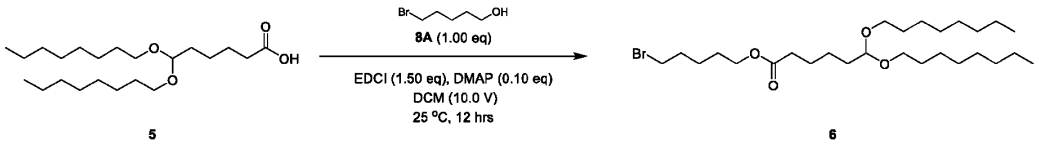


To a solution of compound **9** (8.00 g, 22.9 mmol) in MeCN (80.0 mL) was added compound **9A** (3.97 g, 22.9 mmol) and DIEA (4.44 g, 34.3 mmol) at 25 °C. The mixture was stirred at 25 °C for 2 hrs. LCMS (product RT = 1.37 min) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to give compound **10** (8.00 g, 18.1 mmol, 79.0% yield) as off-white solid.

^{1}H NMR: ET89912-8-P1B (400 MHz, $CDCl_3$)

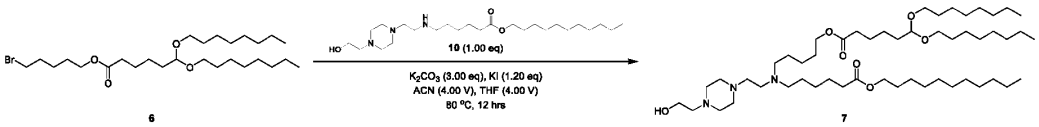
δ 4.05 - 4.08 (m, 2H), 3.67 - 3.70 (m, 2H), 3.42 - 3.44 (m, 1H), 2.90 - 2.99 (m, 2H), 2.54 - 2.62 (m, 11H), 2.33 - 2.35(m, 2H), 1.61 - 1.67 (m, 7H), 1.29 - 1.32 (m, 19 H), 0.88 - 0.91 (m, 3H).

General procedure for preparation of compound 6



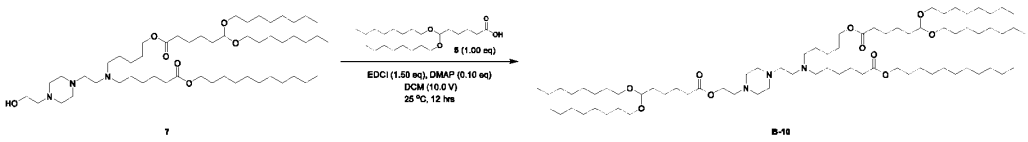
To a solution of compound **5** (2.68 g, 7.18 mmol) and compound **8A** (1.20 g, 7.18 mmol) in DCM (26.8 mL) was added DMAP (87.7 mg, 0.72 mmol) and EDCI (2.07 g, 10.8 mmol) at 25 °C. The mixture was stirred at 25 °C for 12 hrs. LCMS (product RT = 5.28 min) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1) to compound **6** (1.50 g, 2.88 mmol, 40.0% yield) as colorless oil.

General procedure for preparation of compound 7



To a solution of compound **6** (1.50 g, 2.88 mmol) and compound **10** (1.27 g, 2.88 mmol) in THF (6.00 mL) and ACN (6.00 mL) was added K_2CO_3 (1.19 g, 8.63 mmol) and KI (0.57 g, 3.45 mmol) at 25 °C. The mixture was stirred at 80 °C for 12 hrs. LCMS (product RT = 3.68 min) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1) to compound **7** (1.00 g, 1.13 mmol, 39.4% yield) as yellow oil.

General procedure for preparation of compound B-10



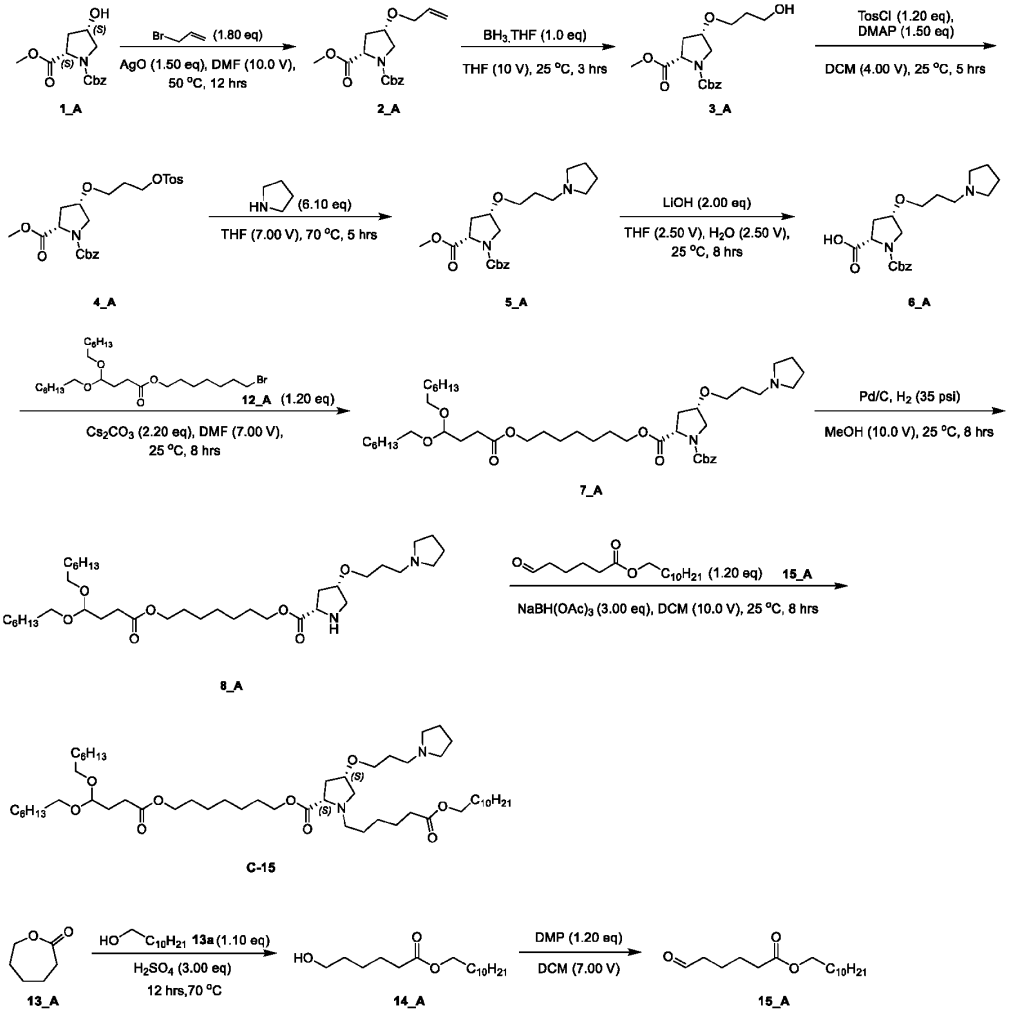
7 B-10

To a solution of compound **7** (1.00 g, 4.45 mmol) and compound **5** (0.42 g, 4.45 mmol) in DCM (1.00 mL) was added EDCI (0.33 g, 1.70 mmol) and DMAP (27.7 mg, 0.23 mmol). The mixture was stirred at 25 °C for 12 hrs. LCMS (product RT = 4.54 min) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove DCM. The residue was purified by pre-HPLC (column: X-Select CSH Phenyl-Hexyl 100*30 5u; mobile phase: [H_2O (0.2%FA) – CAN/THF = 1/1]; gradient: 30% - 70% B over 20.0 min) to give **B-10** (0.39 g, 0.32 mmol, 27.8% yield) as brown oil.

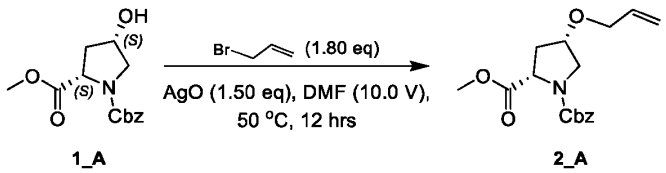
^{1}H NMR: ET89912-34-P1B1 (400 MHz, $CDCl_3$)

δ 4.46 - 4.47 (m, 2H), 4.44 - 4.45 (m, 2H), 4.04 - 4.07 (m, 4H), 3.54 - 3.56 (m, 4H), 3.01 - 3.09 (m, 6H), 2.81 - 2.89 (m, 8H), 2.32 - 2.35 (m, 7H), 1.61 - 1.66 (m, 27H), 1.27 - 1.56 (m, 66H), 0.87 - 0.90 (m, 15H).

Synthesis of s,s C-15



General procedure for preparation of compound 2_A



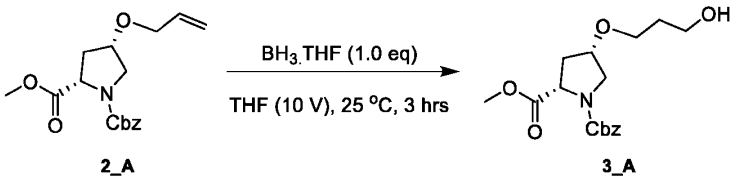
To a solution of compound **1_A** (29.7 g, 106 mmol) in DMF (100 mL) was added Ag_2O (19.8 g, 160 mmol,) and 3-bromoprop-1-ene (23.2 g, 191 mmol) in dark. The mixture was stirred at 50 °C for 12 hrs. LCMS (product RT = 0.502 min) showed the starting material was consumed

completely. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate=10/1 to 4/1). Compound **2_A** (18.3 g, 57.3 mmol, 53.89% yield) was obtained as a colorless oil.

^{1}H NMR: ET89868-64-P1A1 (400 MHz, $CDCl_3$)

δ = 7.28 - 7.42 (m, 5H), 5.77 - 5.91 (m, 1H), 5.06 - 5.28 (m, 4H), 4.41 - 4.54 (m, 1H), 4.06 - 4.14 (m, 1H), 3.93 (br d, J = 4.4 Hz, 2H), 3.26 - 3.76 (m, 4H), 2.20 - 2.41 (m, 2H)

General procedure for preparation of compound 3_A



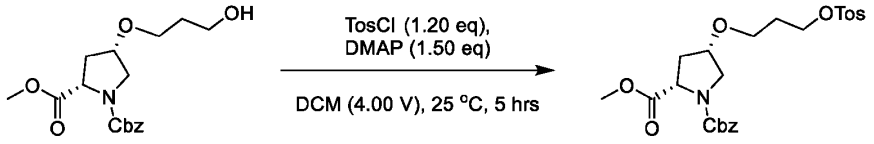
2_A 3_A

To a solution of compound **2_A** (18.3 g, 57.3 mmol) in DMF (100 mL) in THF (200 mL) was added BH3.THF (1 M, 19.8 mL) at 0°C. The mixture was stirred at 0-25 °C for 3 hrs. LCMS (product RT = 1.394 min) showed the starting material was consumed completely. The reaction mixture was quenched by addition NH_4Cl 150 mL at 0 °C, and extracted with EtOAc 600 mL (200 mL x 3). The combined organic layers were washed with brine 200 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate=1/1 to 0/1). Compound **2_A** (7.00 g, 20.8 mmol, 36.2% yield) was obtained as a white oil.

^{1}H NMR: ET89868-65-P1A1 (400 MHz, $CDCl_3$)

δ = 7.28 - 7.41 (m, 5H), 5.05 - 5.24 (m, 2H), 4.42 - 4.26 (m, 1H), 4.01 - 4.08 (m, 1H), 3.60 - 3.79 (m, 8H), 3.50 - 3.58 (m, 2H), 2.12 - 2.34 (m, 2H), 1.76 (quin, J = 5.7 Hz, 2H)

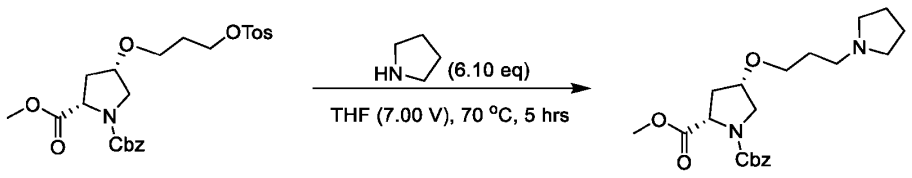
General procedure for preparation of compound 4_A



3_A 4_A

To a solution of compound **3_A** (6.00 g, 17.8 mmol) in DCM (25 mL) was added Et3N (2.70 g, 26.7 mmol), DMAP (109 mg, 889 μmol) and TosCl (4.07 g, 21.3 mmol). The mixture was stirred at 25 °C for 5 hrs. LCMS (product RT = 0.540 min) showed the starting material was consumed completely. The reaction mixture was quenched by addition NH_4Cl 150 mL at 0 °C, and extracted with EtOAc 600 mL (200 mL x 3). The reaction mixture was concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. Compound **4_A** (9.76 g, crude) was obtained as a white solid.

General procedure for preparation of compound 5_A



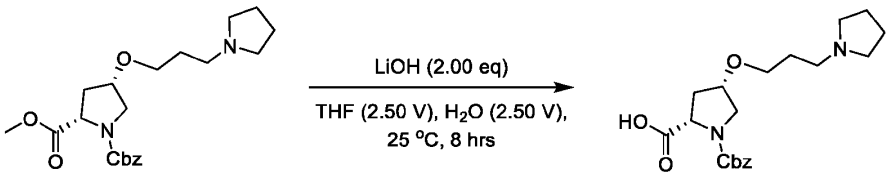
4_A 5_A

To a solution of compound **4_A** (8.74 g, 17.8 mmol in THF (60.0 mL) was added pyrrolidine (7.71 g, 108 mmol). The mixture was stirred at 70 °C for 5 hrs. LCMS (product RT = 0.348 min) showed the starting material was consumed completely. The reaction mixture was diluted with $NaHCO_3$ 50 mL and extracted with EtOAc 240 mL (80 mL x 3). The combined organic layers were washed with brine 50 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, DCM/MeOH = 100/1 to 10/1). Compound **5_A** (5.40 g, 13.8 mmol, 77.8% yield) was obtained as a yellow oil.

^{1}H NMR: ET89868-74-P1A1 (400 MHz, $CDCl_3$)

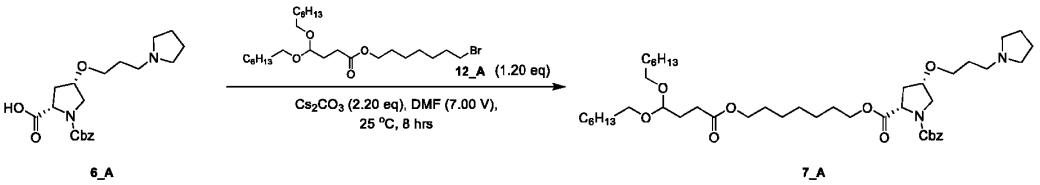
δ = 7.28 - 7.74 (m, 5H), 5.04 - 5.24 (m, 2H), 4.38 - 4.56 (m, 1H), 3.98 – 4.07 (m, 1H), 3.52 - 3.75 (m, 4H), 3.36 - 3.47 (m, 2H), 2.42 - 2.58 (m, 6H), 2.17 - 2.38 (m, 2H), 1.69 - 1.82 (m, 6H)

General procedure for preparation of compound 6_A



To a solution of compound **5_A** (5.40 g, 13.8 mmol) in THF (13.5 mL) and H2O (13.5 mL) was added LiOH (662 mg, 27.7 mmol). The mixture was stirred at 25 °C for 8 hrs. LCMS (product RT = 0.321 min) showed the starting material was consumed completely. The reaction mixture was diluted with H_2O 70 mL and freeze-dried to give a residue. The residue was purified by prep-HPLC (HCl condition). Compound **6_A** (1.20 g, 3.19 mmol, 23.0% yield) was obtained as a yellow oil.

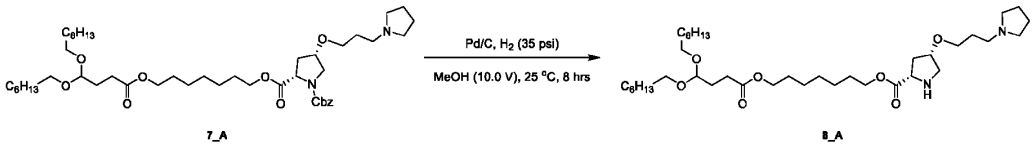
General procedure for preparation of compound 7_A



To a solution of compound **6_A** (400 mg, 1.06 mmol) in DMF (6 mL) was added compound **12_A** (629 mg, 1.28 mmol), Cs_2CO_3 (762 mg, 2.34 mmol) and KI (17.6 mg, 106 μmol). The mixture was stirred at 60 °C for 8 hrs. LCMS (product RT = 0.694 min) showed the starting material was not consumed completely. The reaction mixture was diluted with H_2O 5 mL and extracted with EtOAc 30 mL (10 mL x 3). The combined organic layers were washed with brine 20 mL, dried over Na_2SO_4 filtered and concentrated under reduced pressure to give a residue.

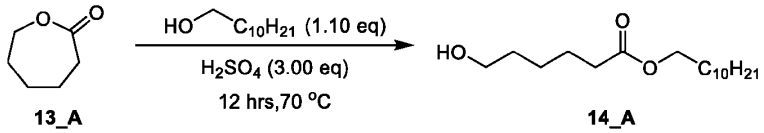
The residue was purified by column chromatography (SiO2, DCM/MeOH =100/1 to 5/1). Compound **7_A** (650 mg, 824 μmol, 77.5% yield) was obtained as a yellow oil.

General procedure for preparation of compound 8_A



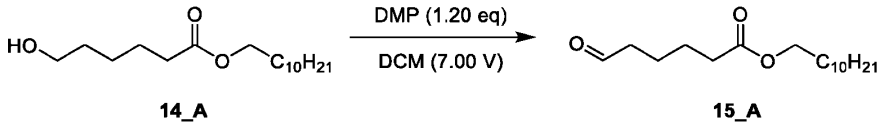
To a solution of compound **7_A** (600 mg, 760 μmol, 1 eq) in THF (10.0 mL) was added Pd/C (1.00 g, 940 μmol, 10% purity) under Ar_2. The suspension was degassed under vacuum and purged with H_2 several times. The mixture was stirred under H_2 (35 psi) at 25°C for 1 hour. LCMS (product RT = 2.876 min) showed the starting material was consumed completely. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. Compound **8_A** (500 mg, crude) was obtained as a yellow oil.

General procedure for preparation of compound 14_A



To a solution of compound **7_A** (10.0 g, 87.6 mmol) in undecan-1-ol (30.2 g, 175 mmol) was added H_2SO_4 (859 mg, 8.76 mmol). The mixture was stirred at 70 °C for 12 hrs. TLC (Petroleum ether/Ethyl acetate = 3/1, product: R_f = 0.27, starting material: R_f = 0.50) showed the starting material was consumed completely. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate=20/1 to 5/1). Compound **14_A** (10.0 g, 34.9 mmol, 39.9% yield) was obtained as colorless oil.

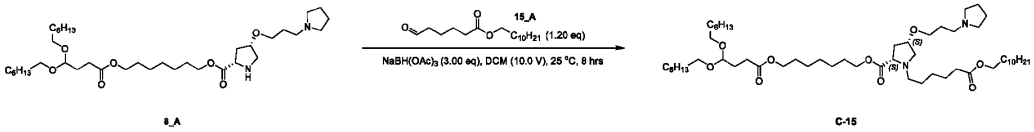
General procedure for preparation of compound 15_A



14_A **15_A**

To a solution of compound **15_A** (5.00 g, 17.5 mmol) in DCM (50.0 mL) was added DMP (8.88 g, 21.0 mmol. The mixture was stirred at 25 °C for 3 hrs. TLC (Petroleum ether/Ethyl acetate = 3/1, product: R_f = 0.22, starting material: R_f = 0.62) showed the starting material was consumed completely. The reaction mixture was quenched by addition $Na_2S_2O_3$ 80 mL at 0 °C, and then extracted with DCM 300 mL (100 mL x 3). The combined organic layers were washed with $NaHCO_3$ 100 mL and brine 100 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate=20/1 to 6/1). Compound **15_A** (4.00 g, 14.1 mmol, 80.57% yield) was obtained as a colorless oil.

General procedure for preparation of C-15



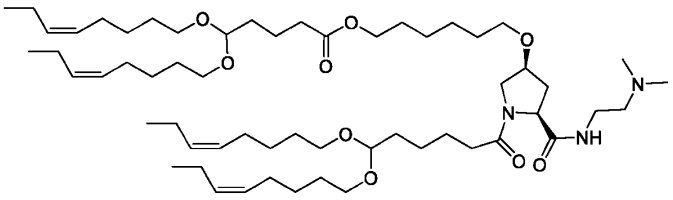
8_A **C-15**

To a solution of compound **8_A** (500 mg, 763 μmol) in DCM (5.00 mL) was added compound **15_A** (261 mg, 916 μmol) and $NaBH(OAc)_3$ (485 mg, 2.29 mmol). The mixture was stirred at 25 °C for 8 hrs. LCMS (product RT = 1.557 min) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove DCM, then washed with H_2O 10 mL, and extracted with EtOAc 90 mL (30 mL x 3). The combined organic layers were washed with $NaHCO_3$ 50 mL and brine 50 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, DCM/MeOH =100/1 to 10/1). **C-15** (416 mg, 450 μmol, 59.01% yield) was obtained as a brown oil.

^{1}H NMR: ET89868-74-P1A1 (400 MHz, $CDCl_3$)

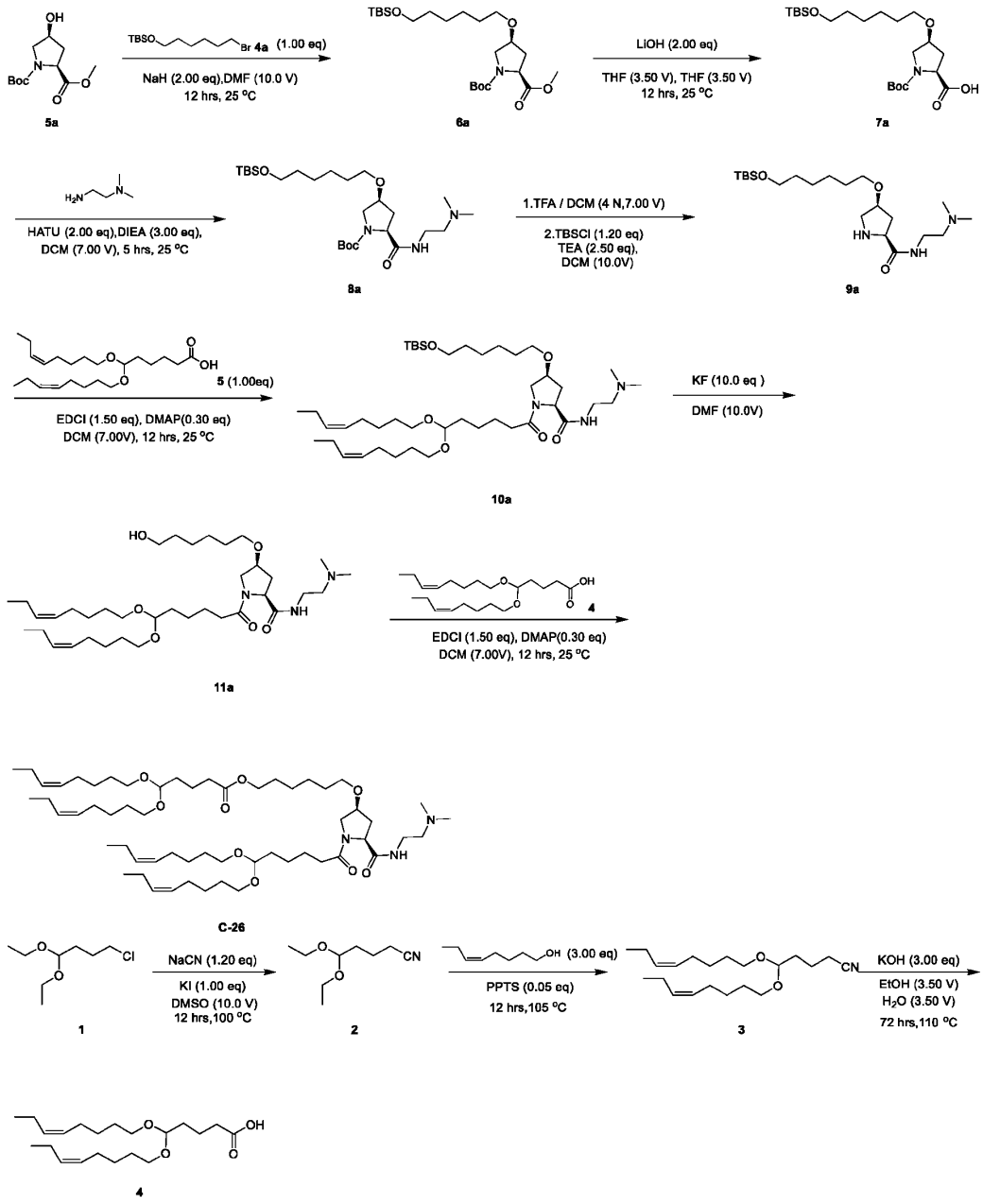
δ = 4.50 (s, 1H), 4.06 (br d, J = 3.0 Hz, 6H), 3.56 (s, 2H), 3.43 (br d, J = 6.5 Hz, 4H), 3.22 (dd, J = 1.9, 10.2 Hz, 1H), 3.09 (s, 1H), 2.59 - 2.80 (m, 6H), 2.22 - 2.48 (m, 8H), 1.88 (br s, 10H), 1.63 (br s, 14H), 1.27 (br s, 40H), 0.89 (s, 9H)

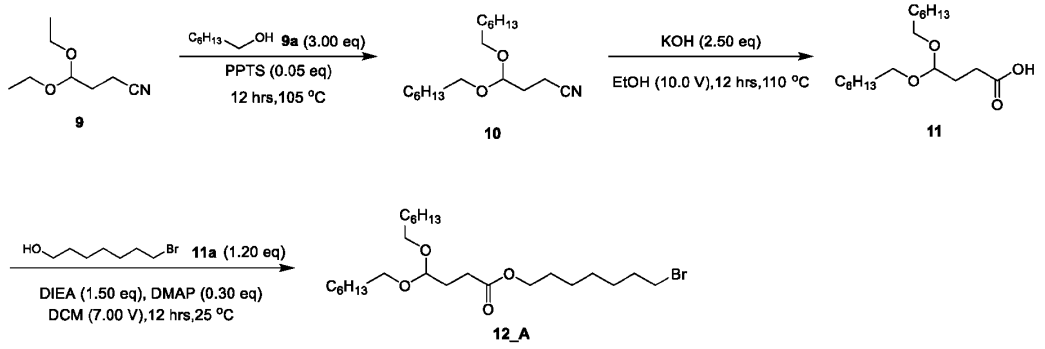
Synthesis of C-26



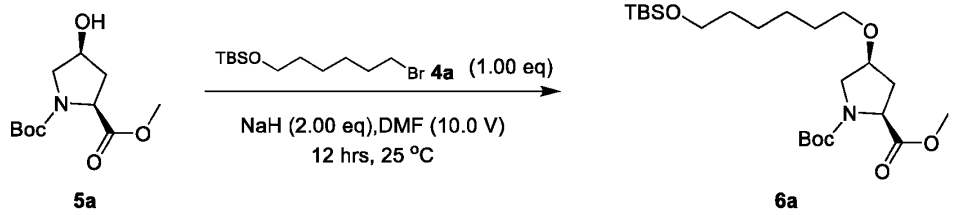
C-26

Synthesis of E-1


5a
4a (1.00 eq)
NaH (2.00 eq),DMF (10.0 V)
12 hrs, 25 °C
6a
LiOH (2.00 eq)
THF (3.50 V), THF (3.50 V)
12 hrs, 25 °C
7a
HATU (2.00 eq),DIEA (3.00 eq),
DCM (7.00 V), 5 hrs, 25 °C
8a
1.TFA / DCM (4 N,7.00 V)
2.TBSCl (1.20 eq)
TEA (2.50 eq),
DCM (10.0V)
9a
5 (1.00eq)
EDCI (1.50 eq), DMAP(0.30 eq)
DCM (7.00V), 12 hrs, 25 °C
10a
KF (10.0 eq)
DMF (10.0V)
11a
4
EDCI (1.50 eq), DMAP(0.30 eq)
DCM (7.00V), 12 hrs, 25 °C
C-26
1
NaCN (1.20 eq)
KI (1.00 eq)
DMSO (10.0 V)
12 hrs,100 °C
2
(3.00 eq)
PPTS (0.05 eq)
12 hrs,105 °C
3
KOH (3.00 eq)
EtOH (3.50 V)
H2O (3.50 V)
72 hrs,110 °C
4



General procedure for preparation of compound 6a

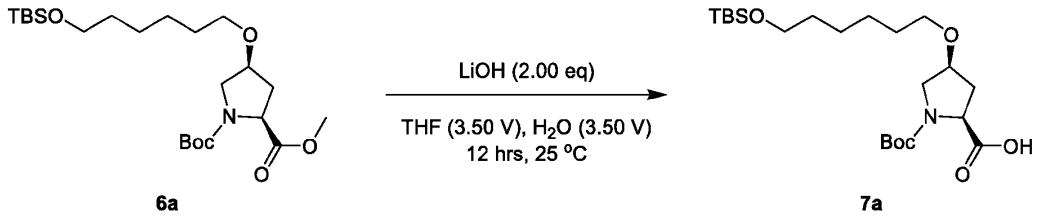


To a solution of compound **5a** (10.0 g, 40.7 mmol) and NaH (2.35 g, 91.4 mmol) in DMF (100 mL) was added compound **4a** (12.0 g, 40.7 mmol). The mixture was stirred at 25 °C for 12 hrs. LCMS (product RT = 4.233) showed the starting material was consumed completely. The reaction mixture was diluted with H_2O (300 mL) and extracted with EtOAc (100 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1). Compound **6a** (6.00 g, 32.0% yield) was obtained as yellow oil.

^{1}H NMR: ET89867-48-P1A1 (400 MHz, $CDCL_3$-d_6)

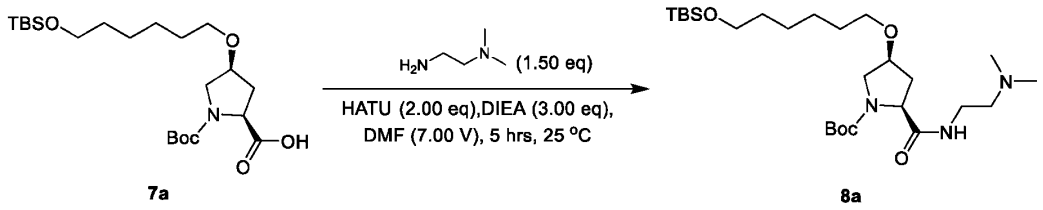
δ 4.26 - 4.46 (m, 1H), 3.95 – 4.10 (m, 1H), 3.67 - 3.78 (m, 3H), 3.57 - 3.62 (m, 3H), 3.31 - 3.48 (m, 3H), 2.20 - 2.34 (m, 1H), 2.00 - 2.09 (m, 1H), 1.48 - 1.53 (m, 3H), 1.46 (d, J = 5.6 Hz, 4H), 1.39 - 1.44 (m, 6H), 1.30 - 1.36 (m, 4H), 0.89 (s, 9H), 0.04 (s, 6H)

General procedure for preparation of compound 7a



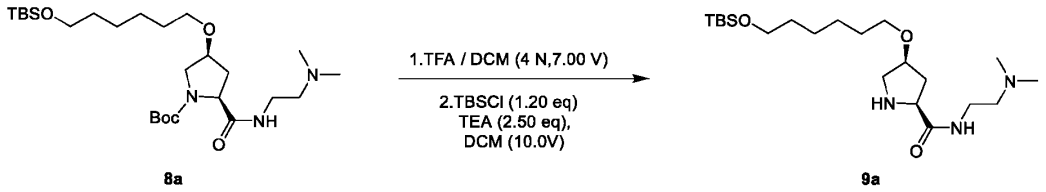
To a solution of compound **6a** (6.00 g, 13.1 mmol) in THF/H_2O (42.0 mL) was added LiOH (1.10 g, 26.2 mmol). The mixture was stirred at 25 °C for 12 hrs. LCMS (product RT = 3.949) showed the starting material was consumed completely. The reaction mixture was adjusted to pH = 6 with sat. $NaHCO_3$ solution. Then the reaction mixture was diluted with H_2O (100 mL) and extracted with EtOAc (50.0 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. Compound **7a** (5.01 g, crude) was obtained as yellow oil.

General procedure for preparation of compound 8a



To a solution of compound **7a** (5.00 g, 11.2 mmol) in DMF (35.0 mL) was added N', N'-dimethylethane-1,2-diamine (1.48 g, 16.8 mmol), HATU (8.53 g, 22.4 mmol) and DIEA (4.35 g, 33.6 mmol). The mixture was stirred at 25 °C for 5 hrs. LCMS (product RT = 2.700) showed the starting material was consumed completely. The reaction mixture was diluted with H_2O (100 mL) and extracted with EtOAc (50.0 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. Compound **7a** (5.01 g, crude) was obtained as yellow oil.

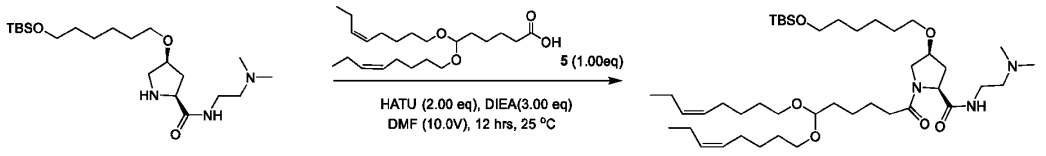
General procedure for preparation of compound 9a



8a 9a

To a solution of compound **8a** (5.01 g, 4.13 mmol) in TFA/DCM(4N,7.00V) (35.0 mL). The mixture was stirred at 25 °C for 1 hr. LCMS (product RT = 1.878) showed the starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove DCM/TFA. Then was added TBSCl (1.38 g, 4.96 mmol), TEA (1.93 g, 10.3 mmol) in DCM (50.0 ml). The mixture was stirred at 25 °C for 1 hr. TLC (petroleum ether/ethyl acetate = 0/1, product: R_f = 0.25, starting material: R_f = 0.14) showed the starting material was consumed completely. The reaction mixture was diluted with H_2O (20.0 mL) and extracted with EtOAc (10.0 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. Compound **3** (3.12 g, crude) was obtained as brown oil.

General procedure for preparation of compound **10a**



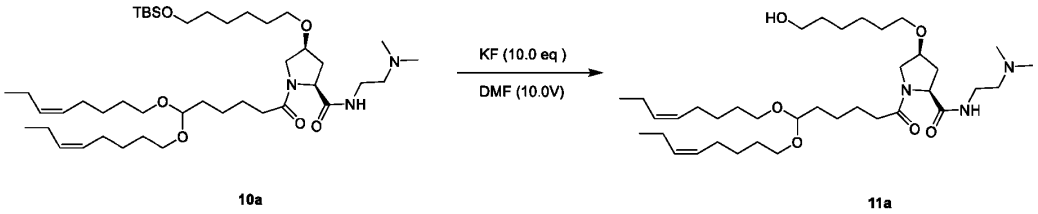
9a 10a

To a solution of compound **9a** (2.60 g, 6.25 mmol) in DMF (26.0 ml) was added compound **5** (2.00 g, 5.63 mmol), HATU (4.76 g, 12.5 mmol) and DIEA (2.43 g, 18.7 mmol). The mixture was stirred at 25 °C for 5 hrs. LCMS (product RT = 3.907) showed the starting material was consumed completely. The reaction mixture was added H_2O (100 ml) at 25 °C and extracted with EtOAc (30 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. Compound **10a** (6.12 g, crude) was obtained as brown oil.

^{1}H NMR: ET89867-73-P1A1 (400 MHz, $CDCL_3$-d_6)

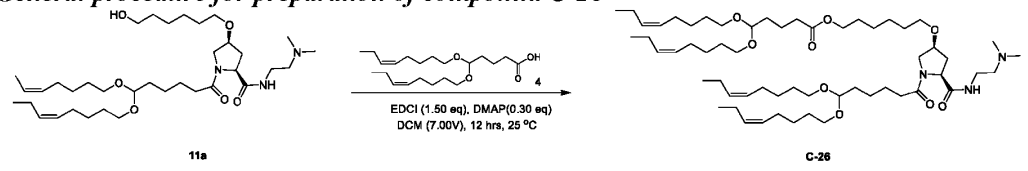
δ 5.27 - 5.43 (m, 4H), 4.39 - 4.59 (m, 2H), 3.94 – 4.07 (m, 1H), 3.73 - 3.83 (m, 1H), 3.54 - 3.62 (m, 4H), 3.35 - 3.46 (m, 6H), 3.20 - 3.30 (m, 3H), 2.18 - 2.32 (m, 6H), 1.98 - 2.11 (m, 10H), 1.71 - 1.91 (m, 6H), 1.52 - 1.64 (m, 10H), 1.37 - 1.45 (m, 6H), 1.30 - 1.34 (m, 4H), 0.93 - 0.98 (m, 6H), 0.88 - 0.90 (m, 8H), 0.00 - 0.11 (m, 6H)

General procedure for preparation of compound 11a



To a solution of compound **10a** (2.50 g, 3.26 mmol) and KF (1.90 g, 32.6 mmol) in DMF (25.0 mL). The mixture was stirred at 105 °C for 16 hrs. LCMS (product RT = 3.039) showed the starting material was consumed completely. The reaction mixture was diluted with H_2O (100 mL) and extracted with EtOAc (50.0 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. Compound **2A** (2.20 g, crude) was obtained as yellow oil.

General procedure for preparation of compound C-26



To a solution of compound **11a** (1.00 g, 1.53 mmol) in DCM (7.00 ml) was added compound **4** (0.54 g, 1.37 mmol), EDCI (0.40 g, 2.29 mmol), DMAP (0.05 g, 0.46 mmol). The mixture was stirred at 25 °C for 5 hrs. LCMS (product RT = 4.329) showed the starting material was consumed completely. The reaction mixture was added H_2O (100 ml) at 25 °C and extracted with EtOAc (30 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and

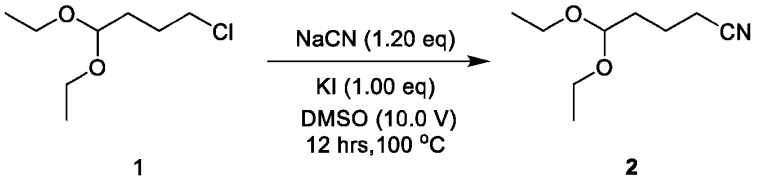
concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1). Compound **10a** (0.52 g, 0.53 mmol, 34.5% yield) was obtained as yellow oil.

LCMS:ET89867-78-P1A8 product: RT =2.387 min, $[M+H]^+$ = 988, purity = 98.1%

^{1}H NMR: ET89867-15-P1A3 (400 MHz, DMSO-d6)

δ 5.26 - 5.41 (m, 8H), 4.50 - 4.59 (m, 1H), 4.42 - 4.49 (m, 2H), 4.19 - 4.36 (m, 1H), 4.01 - 4.10 (m, 2H), 3.50 - 3.63 (m, 5H), 3.35 - 3.45 (m, 7H), 2.56 - 2.73 (m, 2H), 2.43 - 2.53 (m, 2H), 2.25 - 2.38 (m, 8H), 2.18 - 2.24 (m, 2H), 2.97 - 1.09 (m, 18H), 1.53 - 1.75 (m, 20H), 1.31 - 1.46 (m, 14H), 0.95 (t, J = 7.6 Hz, 12H)

General procedure for preparation of compound 2



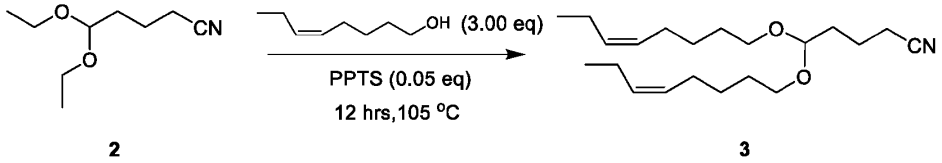
1 2

To a solution of compound **1** (90.0 g, 498 mmol), KI (82.7 g, 498 mmol) and NaCN (29.67 g, 605 mmol) in DMSO (900 mL). The mixture was stirred at 100 °C for 12 hrs. TLC (petroleum ether/ ethyl acetate = 5/1, product: R_f = 0.60, starting material: R_f = 0.56) showed the starting material was consumed completely. The reaction mixture was adjusted to pH = 8 with sat. $NaHCO_3$ solution. Then was diluted with H_2O (1.00 L) and extracted with EtOAc (500 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1). Compound **6a** (40.0 g, 233 mmol 32.0% yield) was obtained as yellow oil.

^{1}H NMR: ET89867-7-P1A2 (400 MHz, CDCL3-d6)

δ 4.50 (br s, 1H), 3.59 - 3.71 (m, 2H), 3.43 - 3.55 (m, 2H), 2.35 - 2.46 (m, 2H), 1.75 (br s, 4H), 1.20 (t, J = 7.0 Hz, 6H)

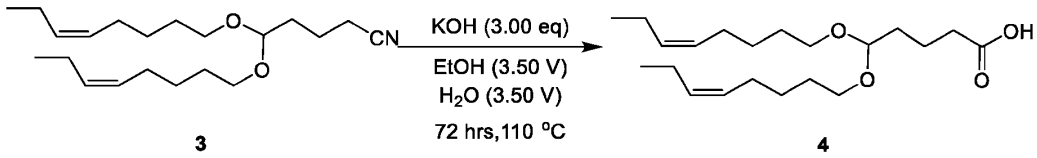
General procedure for preparation of compound 3



2 3

To a solution of compound **2** (22.5 g, 131 mmol), (Z)-oct-5-en-1-ol (50.5 g, 394 mmol) and PPTS (1.65 g, 6.57 mmol). The mixture was stirred at 105 °C for 12 hrs. TLC (petroleum ether/ ethyl acetate = 5/1, product: R_f = 0.78, starting material: R_f = 0.60) showed the starting material was consumed completely. Concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1). Compound **3** (32.0 g, 95.2 mmol 32.0% yield) was obtained as yellow oil.

General procedure for preparation of compound 4



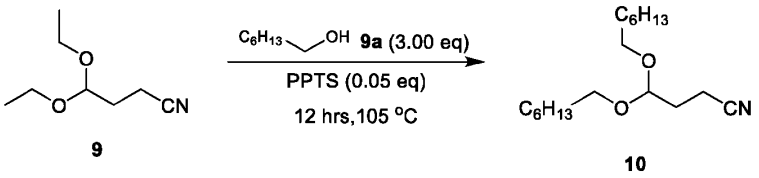
3 4

To a solution of compound **3** (31.0 g, 92.4 mmol), KOH (18.2 g, 323 mmol) in EtOH/H_2O (217 ml). The mixture was stirred at 105 °C for 72 hrs. TLC (petroleum ether/ ethyl acetate = 3/1, product: R_f = 0.32, starting material: R_f = 0.90) showed the starting material was consumed completely. Concentrated under reduced pressure to give a residue. Compound **4** (30.0 g, 84.6 mmol 91.5% yield) was obtained as yellow oil.

^{1}H NMR:ET89867-23-P1A1 (400 MHz, CDCL3-d6)

δ 5.25 - 5.42 (m, 4H), 4.38 - 4.49 (m, 1H), 3.48 - 3.58 (m, 2H), 3.37 - 3.44 (m, 2H), 2.10 - 2.19 (m, 2H), 1.97 – 2.09 (m, 8H), 1.49 - 1.62 (m, 8H), 1.34 - 1.45 (m, 4H), 0.95 (t, J = 7.6 Hz, 6H)

General procedure for preparation of compound 10



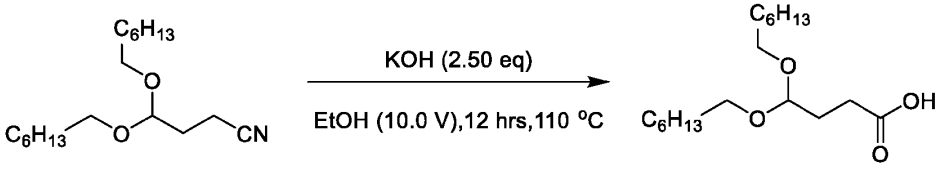
9 10

To a solution of compound **9** (11.4 g, 72.5 mmol), compound **9a** (33.7 g, 290 mmol) and PPTS (1.82 g, 3.62 mmol). The mixture was stirred at 105 °C for 12 hrs. TLC (petroleum ether/ ethyl acetate = 5/1, product: R_f = 0.78, starting material: R_f = 0.60) showed the starting material was consumed completely. Concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1). Compound **3** (14.0 g, 47.0 mmol 64.9% yield) was obtained as yellow oil.

^{1}H NMR: ET89867-27-P1A1 (400 MHz, CDCL3-d6)

δ 3.55 - 3.65 (m, 2H), 3.38 - 3.48 (m, 2H), 2.42 (t, J = 7.4 Hz, 2H), 1.88 – 2.01 (m, 2H), 1.54 - 1.63 (m, 4H), 1.24 - 1.40 (m, 16H), 0.82 - 0.95 (m, 6H)

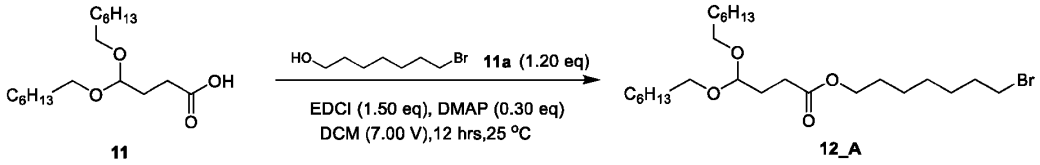
General procedure for preparation of compound 11



10 11

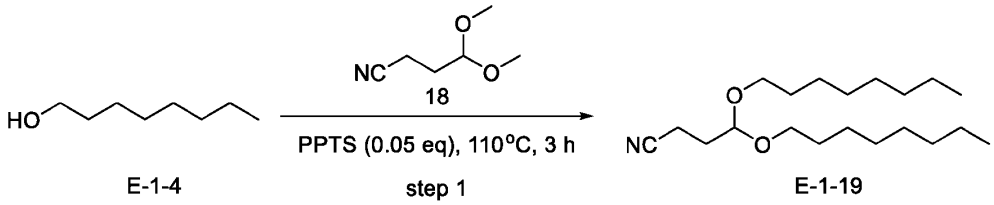
To a solution of compound **10** (12.0 g, 40.3 mmol), KOH (7.92 g, 141 mmol) in EtOH (120 ml). The mixture was stirred at 105 °C for 12 hrs. TLC (petroleum ether/ ethyl acetate = 3/1, product: R_f = 0.32, starting material: R_f = 0.8) showed the starting material was consumed completely. Concentrated under reduced pressure to give a residue. Compound **4** (11.3 g,crude) was obtained as yellow oil.

General procedure for preparation of compound 12_A



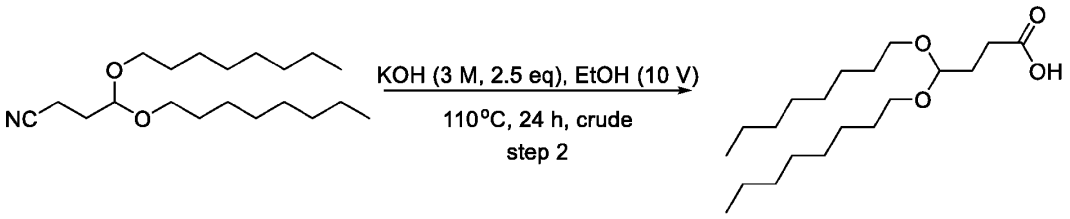
To a solution of compound **11** (11.0 g, 34.7 mmol) in DCM (77.0 ml) was added compound **11a** (6.10 g, 31.2 mmol), EDCI (9.99 g, 52.1 mmol), DMAP (0.84 g, 6.95 mmol). The mixture was stirred at 25 °C for 12 hrs. TLC (petroleum ether/ethyl acetate = 0/1, product: R_f = 0.60, starting material: R_f = 0.32) showed the starting material was consumed completely. The reaction mixture was added H_2O (100 ml) at 25 °C and extracted with EtOAc (30 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1). Compound **10a** (5.80 g, 11.75 mmol, 33.8% yield) was obtained as yellow oil.

Synthesis of E-1-19



Into a 100 mL around bottom was added octanol (5 g, 38.393 mmol, 1 equiv), 4,4-dimethoxybutanenitrile (2.23 g, 17.277 mmol, 0.45 equiv) and PPTS (0.48 g, 1.920 mmol, 0.05 equiv) at room temperature. The resulting mixture was stirred for 3 h at 110°C. The mixture was allowed to cool down to room temperature. The mixture was allowed to cool down to room temperature. The resulting mixture was diluted with heptane (150 mL). The resulting mixture was washed with 2 x 100 mL of water, 1x100 mL of sat. $NaHCO_3$ and 100 mL of brine. The resulting mixture was dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford 4,4-bis(octyloxy)butanenitrile (8.0 g, crude) as a yellow oil. The crude product was used in the next step directly without further purification.

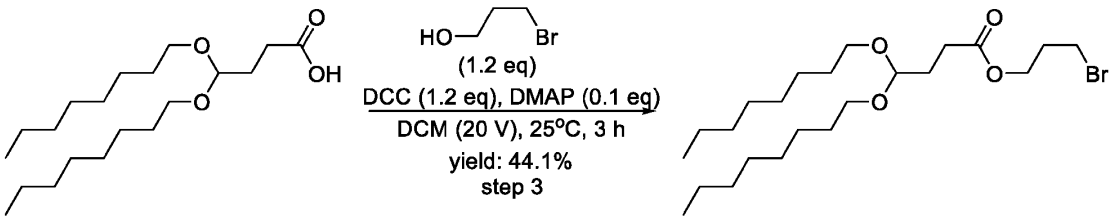
Synthesis of PH-AERA-SDPC-2024-01-E-1-20



E-1-19 E-1-20

Into a 250 mL pressure tank reactor was added 4,4-bis(octyloxy)butanenitrile (8 g, 24.592 mmol, 1 equiv), EtOH (80 mL) and aqueous solution of KOH (3 M, 20 mL, 61.482 mmol, 2.5 equiv) at room temperature. The resulting mixture was stirred for 24 h at 110°C. The mixture was allowed to cool down to room temperature. The resulting mixture was concentrated under reduced pressure. The resulting mixture was diluted with water (50 mL). The mixture was acidified to pH 5 with 1M HCl (aq.). The resulting mixture was extracted with EtOAc (2 x 200 mL). The combined organic layers were washed with water (3 x 100 mL) and brine (2 x 100 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford 4,4-bis(octyloxy)butanoic acid (5.2 g, crude). The crude product was used in the next step directly without further purification.

Synthesis of E-1-57

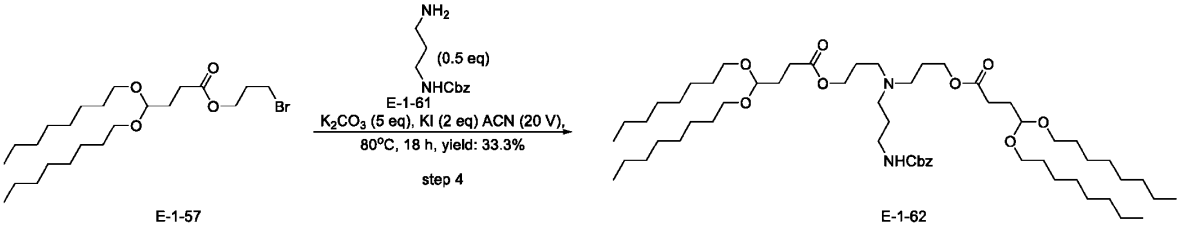


E-1-20 E-1-57

Into a 250 mL 3-necked round-bottom flask were added 4,4-bis(octyloxy)butanoic acid (5.2 g, 15.093 mmol, 1 equiv), 3-bromopropanol (2.52 g, 18.112 mmol, 1.2 equiv), DCM (104 mL) and DMAP (0.18 g, 1.509 mmol, 0.1 equiv) at 25°C. To the above mixture was added N-(N-cyclohexylcarboximidoyl)cyclohexanamine (3.74 g, 18.112 mmol, 1.2 equiv) in portions at room temperature. The resulting mixture was stirred 3 h at room temperature. The resulting mixture was diluted with n-heptane (100 mL). The resulting mixture was filtered. The filtrate was

concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with n-heptane / EA (98:2) to afford 3-bromopropyl 4,4-bis(octyloxy)butanoate (3.1 g, yield: 44.1%) as a colorless oil.

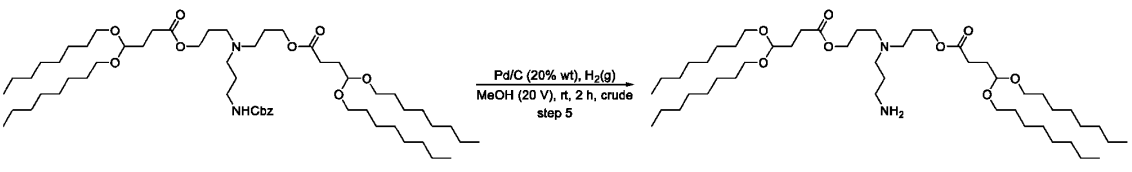
Synthesis of E-1-62



E-1-57 E-1-62

Into a 100 mL 3-necked round-bottom flask were added 3-bromopropyl 4,4-bis(octyloxy)butanoate (3.0 g, 6.445 mmol, 1 equiv), benzyl N-(3-aminopropyl)carbamate (0.67 g, 3.223 mmol, 0.5 equiv), K_2CO_3 (4.45 g, 32.225 mmol, 5 equiv), MeCN (60 mL) and KI (2.14 g, 12.890 mmol, 2 equiv) at room temperature. The resulting mixture was stirred for 18 h at 80 °C under nitrogen atmosphere. The mixture was allowed to cool down to room temperature. The resulting mixture was diluted with water (50 mL). The resulting mixture was extracted with n-heptane (3 x 80 mL). The combined organic layers were washed with water (2 x 100 mL), MeOH/water (1:3, 3 x 100 mL) and brine (100 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with heptane / EA (3:7) to afford 3-[(3-{[(benzyloxy)carbonyl]amino}propyl)(3-{[4,4-bis(octyloxy)butanoyl]oxy}propyl)amino]propyl 4,4-bis(octyloxy)butanoate (2.1 g, yield: 33.3%) as a yellow oil.

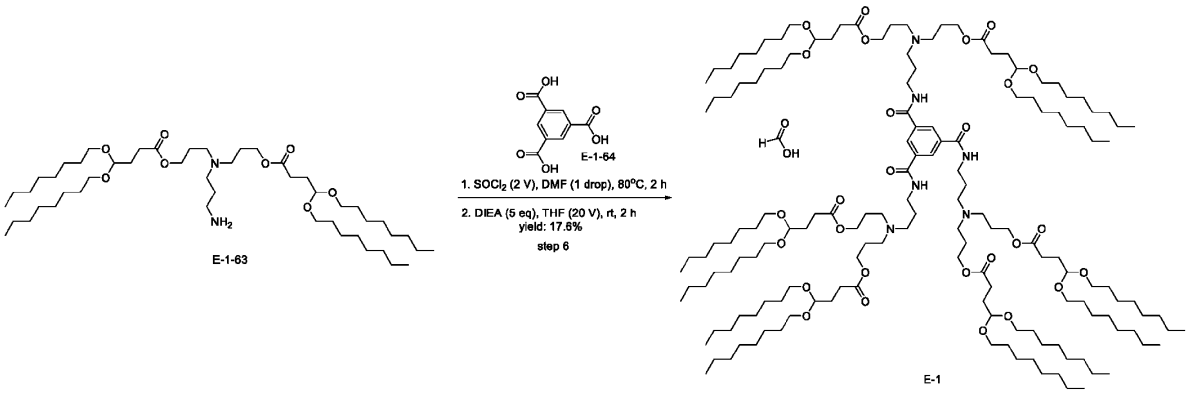
Synthesis of E-1-63



E-1-62 E-1-63

Into a 100 mL round-bottom flask was place 3-[(3-{[(benzyloxy)carbonyl]amino}propyl)(3-{[4,4-bis(octyloxy)butanoyl]oxy}propyl)amino]propyl 4,4-bis(octyloxy)butanoate (1.5 g, 1.535 mmol, 1 equiv) and methanol (30 mL) at room temperature. To this was added Pd/C (300 mg, 20% wt) at room temperature. The mixture was stirring for 2 hours at room temperature under H_2 (25 atm) atmosphere. The resulting mixture was filtered, the filter cake was washed with EA (2 x 15 mL). The filtrate was concentrated under reduced pressure to afford 3-[(3-aminopropyl)(3-{[4,4-bis(octyloxy)butanoyl]oxy}propyl)amino]propyl 4,4-bis(octyloxy)butanoate (900 mg, crude). The crude product was used in the next step directly without further purification.

Synthesis of E-1



Into a 8 mL vial, was place trimesic acid (50 mg, 0.238 mmol, 1.00 equiv) and Thionyl chloride (100 uL) at room temperature under N_2 atmosphere. The mixture was stirring for 2 hours at 80°C. The resulting mixture was concentrated under reduced pressure.

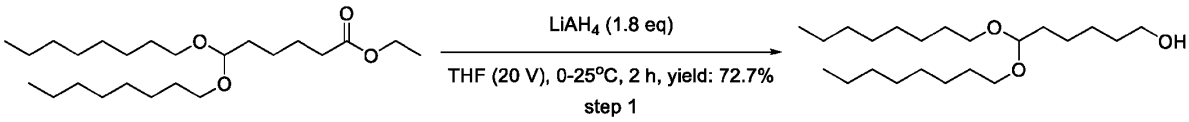
Into another 100 mL round-bottom flask, was place 3-[(3-aminopropyl)(3-{[4,4-bis(octyloxy)butanoyl]oxy}propyl)amino]propyl 4,4-bis(octyloxy)butanoate (882.89 mg, 1.047 mmol, 4.40 equiv), THF (17.7 mL) and DIEA (0.21 mL, 1.190 mmol, 5 equiv) at room temperature. To this was added above acyl chloride (in 2 mL THF) at 0°C. The mixture was stirring for 2 hours at room temperature. The resulting mixture was concentrated under reduced pressure. The residue was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN/IPA (1/1) in H_2O (0.1% FA), 50% to

90% gradient in 10 min; 90% to 90% 5 min detector, UV 205 nm. The fractions were lyophilized directly to afford AERA-E-1-0 (112.5 mg, yield: 17.6%) as light yellow oil.

LCMS-PH-AERA-SDPC-2024-01-E-1-0 (ES, m/z): 2685.2 $[M+H]^+$

^{1}HNMR-PH- AERA-SDPC-2024-01-E-1-0 (300 MHz, $CDCl_3$, *ppm*): δ 8.669 (brs, 3H), 8.381 (brs, 1H), 8.159 (brs, 2H), 4.503 (t, J = 5.7 Hz, 6H), 4.147 (t, J = 6.0 Hz, 12H), 3.613-3.583 (m, 18H), 3.456-3.636 (m, 12H), 3.078-2.784 (m, 20H), 2.401 (t, J = 7.5 Hz, 12H), 2.376-1.894 (m, 30H), 1.548 (t, J = 6.6 Hz, 24H), 1.402-1.148 (m, 120H), 0.918-0.852 (m, 36H).

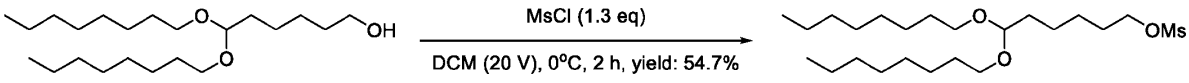
Synthesis of B-13-15



6 15

Into a 100 mL 3-neck bottom flask, was place ethyl 6,6-bis(octyloxy)hexanoate (3 g, 7.488 mmol, 1 equiv) and THF (60 mL) at room temperature. To this was added Lithium aluminum hydride (2.0 M in THF) (6.74 mL, 13.478 mmol, 1.8 equiv) at 0°C under N_2 atmosphere. The mixture was stirring for 2 hours at 25°C. The reaction was quenched by the addition of water (0.51 mL) and NaOH (15%, 0.51 mL) and water (1.55 mL) at 5°C. The resulting $MgSO_4$ was dried. The solid was filtered out, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with Heptane/EA (10/1) to afford 6,6-bis(octyloxy)hexan-1-ol (1.95 g, yield: 72.7%) as a light yellow oil.

Synthesis of B-13-16

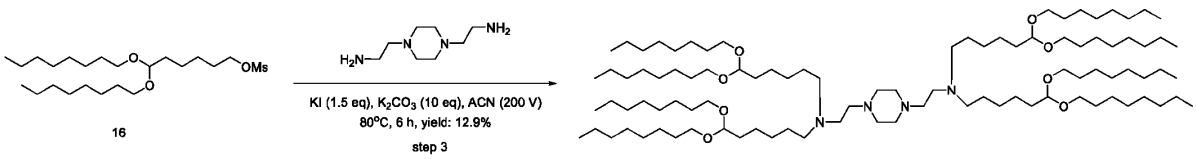


15 16

Into a 250 mL round-bottom flask, was place 6,6-bis(octyloxy)hexan-1-ol (1.95 g, 5.438 mmol, 1 equiv), Et_3N (1.10 g, 10.876 mmol, 2 equiv) and DCM (40 mL) at room temperature. To this was added methanesulfonyl chloride (0.81 g, 7.069 mmol, 1.3 equiv) dropwise at 0°C under N_2 atmosphere. The mixture was stirring for 2 hours at 0°C. The resulting mixture was diluted with DCM (150 mL). The mixture was washed with water (2 x 80 mL) and brine (100 mL). dried over

anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford 6,6-bis(octyloxy)hexyl methanesulfonate (1.3 g, yield: 54.7%). The crude product was used in the next step directly without further purification.

Synthesis of B-13

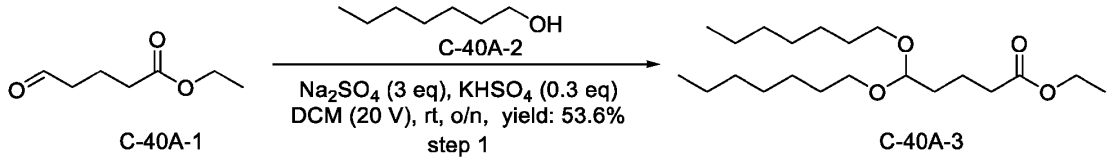


Into a 250 mL round-bottom flask, was place 6,6-bis(octyloxy)hexyl methanesulfonate (1140.67 mg, 2.610 mmol, 4.5 equiv) 2-[4-(2-aminoethyl)piperazin-1-yl]ethanamine (100 mg, 0.580 mmol, 1.00 equiv) and MeCN (20 mL) at room temperature. to this was added KI (144.54 mg, 0.870 mmol, 1.5 equiv) and K_2CO_3 (802.3 mg, 4.640 mmol, 10 equiv) at room temperature under N_2 atmosphere. The mixture was stirring for 6 hours at 80°C. The resulting mixture was concentrated under reduced pressure. The residue was re-purified by silica gel column chromatography, eluted with MeOH/DCM (10/1) to afford final product (138.2 mg, Purity: 94.0%). purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, FA in ACN/IPA=1/1, 40% to 90% gradient in 22 min; detector, ELSD. The distillate was basified to pH = 10 with Na_2CO_3 (1%, aq.) and was concentrated under reduced pressure. The product was dissolved in n-heptane (400 mL) and washed with MeOH/H_2O (4:1) (2x100 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford AERA-B-13-0 (115.3 mg, yield: 12.9%) as a light yellow oil.

LCMS-PH-AERA-SDPC-2024-01-B-13-0 (ES, m/z): 1534.5 $[M+H]^+$

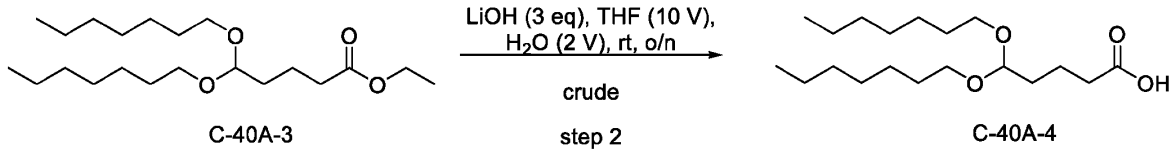
HNMR-PH- AERA-SDPC-2024-01-B-13-0 (300 MHz, CDCl3): δ 4.467 (t, J = 5.7 Hz, 4H), 3.609-3.556 (m, 8H), 3.534-3.377 (m, 8H), 2.559-2.321 (m, 24H), 1.765-1.715 (m, 24H), 1.649-1.534 (m, 104H), 0.92- 0.822 (m, 24H).

Synthesis of C-40A-3



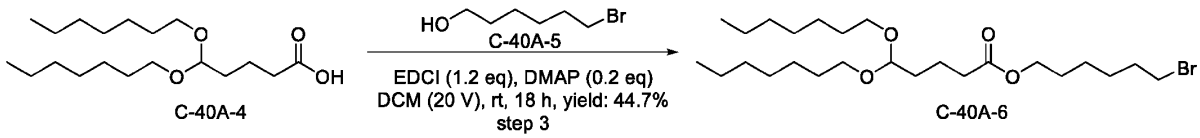
Into a 500 mL round-bottom flask were added ethyl 5-oxopentanoate (6 g, 41.618 mmol, 1 equiv), DCM (120 mL) and Na_2SO_4 (17.73 g, 124.824 mmol, 3.00 equiv) at room temperature. The resulting mixture was stirred at 25°C for 5 min under nitrogen atmosphere. To the above mixture was added heptanol (14.51 g, 124.854 mmol, 3 equiv) and $KHSO_4$ (1.70 g, 12.485 mmol, 0.3 equiv) at 25°C. The resulting mixture was stirred at room temperature for overnight. The resulting mixture was filtered, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with n-heptane / EA (20:1) to afford ethyl 5,5-bis(heptyloxy)pentanoate (8 g, yield: 53.6%) as a colorless oil.

Synthesis of C-40A-4



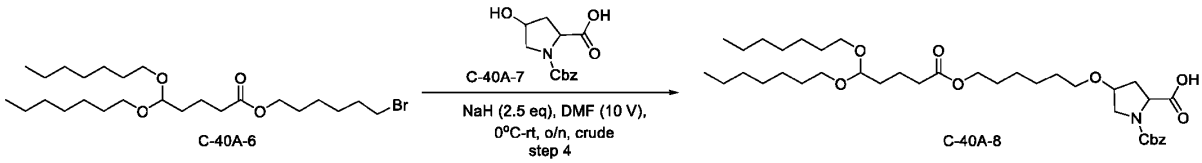
Into a 250 mL round-bottom flask were added ethyl 5,5-bis(heptyloxy)pentanoate (8 g, 22.311 mmol, 1 equiv), THF (80 mL), H_2O (16 mL) and LiOH (1.6 g, 44.693 mmol, 3 equiv) at room temperature. The resulting mixture was stirred at room temperature for 18 h under nitrogen atmosphere. THF was concentrated under vacuum. The mixture was acidified to Ph (4-5) with HCl (0.5M, aq). The aqueous layer was extracted with EtOAc (2 x 200 mL). The organic phase was dried with anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford 5,5-bis(heptyloxy)pentanoic acid (6.0 g, crude). The crude product was used to the next step directly without further purification.

Synthesis of C-40A-6



Into a 250 mL round-bottom flask were added 5,5-bis(heptyloxy)pentanoic acid (6 g, 18.154 mmol, 1 equiv), DCM (120 mL), 6-bromo-1-hexanol (3.29 g, 18.154 mmol, 1 equiv), DMAP (0.44 g, 3.631 mmol, 0.20 equiv) and EDCI (3.38 g, 21.785 mmol, 1.2 equiv) at room temperature. The resulting mixture was stirred at room temperature for 18 h under nitrogen atmosphere. The resulting mixture was washed with 3 x 50 mL of water and 1 x 50 mL of brine. The organic phase was dried with anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with n-heptane / EA (20:1) to afford 6-bromohexyl 5,5-bis(heptyloxy)pentanoate (4 g, yield: 44.7%) as a light yellow oil.

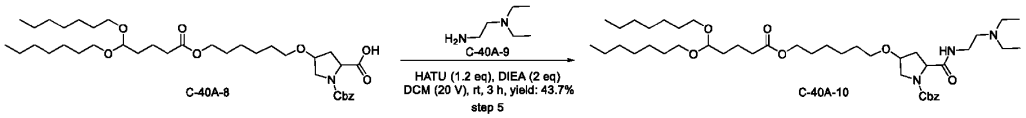
Synthesis of C-40A-8



To a stirred solution of 1-[(benzyloxy)carbonyl]-4-hydroxypyrrolidine-2-carboxylic acid (2.15 g, 8.104 mmol, 1 equiv) and DMF (40 mL) was added NaH (0.49 g, 20.260 mmol, 2.5 equiv) in portions at 0°C under nitrogen atmosphere. The resulting mixture was stirred at 0°C for 30 min under nitrogen atmosphere. To the above mixture was added 6-bromohexyl 5,5-bis(heptyloxy)pentane ate (4 g, 8.104 mmol, 1 equiv) dropwise at 0°C. The resulting mixture was stirred at room temperature for overnight. The reaction was poured into water/ice (100 mL). The aqueous layer was extracted with EtOAc (3 x 100 mL). The organic phase was washed with 3 x 100 mL of water and 1 x 100 mL of brine. The organic phase was dried with anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford 1-

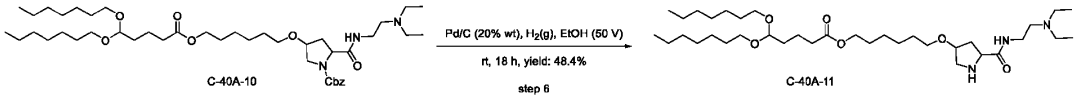
[(benzyloxy)carbonyl]-4-[(6-{[5,5-bis(heptyloxy)pentanoy l]oxy}hexyl)oxy]pyrrolidine-2-carboxylic acid (4.0 g, crude). The crude product was used in the next step directly without further purification.

Synthesis of C-40A-10



Into a 250 mL round-bottom flask were added 1-[(benzyloxy)carbonyl]-4-[(6-{[5,5-bis(heptyloxy)pentanoy l]oxy}hexyl)oxy]pyrrolidine-2-carboxylic acid (4 g), DCM (80 mL), DIEA (1.53 g, 11.800 mmol, 2 equiv), HATU (2.69 g, 7.080 mmol, 1.2 equiv) and (2-aminoethyl)diethylamine (0.69 g, 5.900 mmol, 1 equiv) at room temperature. The resulting mixture was stirred at room temperature for overnight under nitrogen atmosphere. The resulting mixture was washed with 3 x 50 mL of water and 1x50 mL of brine. The organic phase was dried with anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with DCM / MeOH (10:1) to afford benzyl4-[(6-{[5,5-bis(heptyloxy)pentanoyl]oxy}hexyl)oxy]-2-{[2-(diethylamino)ethyl]carbamoyl}pyr rolidine-1-carboxylate (2 g) as a light yellow oil.

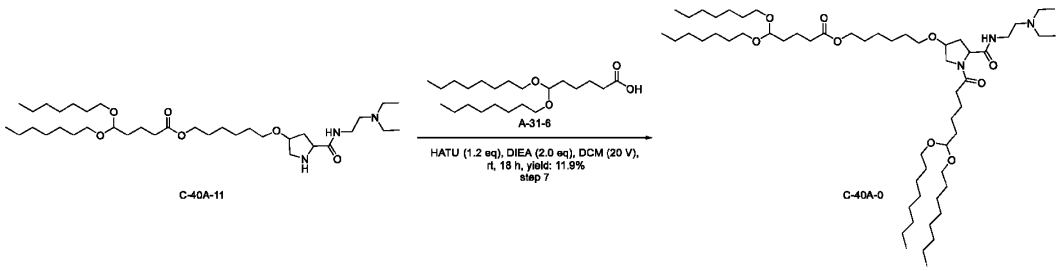
Synthesis of C-40A-11



To a solution of benzyl 4-[(6-{[5,5-bis(heptyloxy)pentanoyl]oxy}hexyl)oxy]-2-{[2-(diethylamino)ethyl]carb amoyl}pyrrolidine-1-carboxylate (2 g, 2.577 mmol, 1 equiv) in 100 mL of EtOH was added Pd/C (0.4 g, 20% wt) under nitrogen atmosphere in a 500 mL round-bottom flask. The mixture was hydrogenated at room temperature for 18 h under hydrogen atmosphere using a hydrogen balloon. The mixture was filtered through a Celite pad and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column

chromatography, eluted with DCM / MeOH (10:1) to afford 6-[(5-{[2-(diethylamino)ethyl]carbam oyl}pyrrolidin-3-yl)oxy]hexyl5,5-bis(heptylox y)pentanoate (0.8 g, yield: 48.6%) as a light yellow oil.

Synthesis of C-40A-0



Into a 100 mL round-bottom flask were added 6,6-bis(octyloxy)hexanoic acid (464.30 mg, 1.246 mmol, 1.00 equiv), DCM (16 mL), DIEA (322.12 mg, 2.492 mmol, 2.00 equiv), HATU (568.59 mg, 1.495 mmol, 1.2 equiv) and 6-[(5-{[2-(diethylamino)ethyl]carbamoyl}pyrrolidin-3-yl)oxy]hexyl 5,5-bis(heptyloxy)pentanoate (800.00 mg, 1.246 mmol, 1 equiv) at room temperature. The resulting mixture was stirred at room temperature C for 18 h under nitrogen atmosphere. The resulting mixture was concentrated under vacuum. The residue was dissolved in n-heptane (300 mL). The resulting mixture was washed with 2 x 50 mL of MeOH / H_2O = 4 / 1. The organic phase was dried with anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by reversed-phase flash chromatography with the following conditions: column, XB-phenyl; mobile phase, MeCN in H_2O (0.1 % FA), 50 % to 90 % gradient in 16 min; detector, UV 200 nm. The fraction was basified to pH = 8 with saturated Na_2CO_3 (1.0%, aq) and concentrated to remove MeCN. The residue was dissolved in n-heptane (100 mL). The mixture was washed with MeOH / H_2O (4 / 1, 2 x 20 mL) and water (1 x 50 mL). The organic phase was dried with anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford 6-({1-[6,6-bis(octyloxy)hexanoyl]-5-{[2-(diethylamino)ethyl]carbamoyl}pyrrolidin-3-yl}oxy)hexyl5,5-bis(heptyloxy)pentanoate (147.9 mg, yield: 11.9%) as light yellow oil.

LC-MS-PH-AERA-SDPC-2024-01-C-40A-0: (ES, m/z): 996.8 $[M+H]^+$;

^{1}H-NMR-PH-AERA-SDPC-2024-01-C-40A-0: (300 MHz, $CDCl_3$, *ppm*): δ 4.491-4.474 (m, 1H), 4.460-4.442 (m, 2H), 4.233-4.200 (m, 1H), 4.065 (t, *J* = 6.9 Hz, 2H), 3.750 (s, 1H), 3.606-3.523 (m, 4H), 3.465-3.389 (m, 7H), 3.366-3.250 (m, 2H), 2.544-2.504 (m, 5H), 2.360-2.314 (m, 4H), 2.011 (s, 1H), 1.728-1.523 (m, 20H), 1.456-1.289 (m, 44H), 1.082 (s, 6H), 0.891 (t, *J* = 6.3 Hz, 12H).

Table X. Exemplary compositions described herein and properties thereof

Composition	Ionizable Lipid	IL mole %	DSPC mole %	Chol mole %	DMG-PEG mole %	DSPE-PEG-Fab mole %	DSPE-PEG-Azide mole %	Fab source	Particle Size	PDI	EE	mRNA	Dose (mg/kg)	% GFP + T cells
1	A-6	33.0	39.9	25.4	1.54	0.05	0.11	Fab1	97.42	0.0901	92	GFP	1	***
2	A-31	33.0	39.9	25.4	1.54	0.05	0.11	Fab1	102.1	0.1182	91	GFP	1	**
3	B-13	33.0	39.9	25.4	1.54	0.05	0.11	Fab1	81.96	0.1073	88	GFP	1	*
5	C-40	33.0	39.9	25.4	1.54	0.05	0.11	Fab1	130.1	0.1152	99	GFP	1	*
6	E-1	33.0	39.9	25.4	1.54	0.05	0.11	Fab1	78.43	0.0410	95	GFP	1	*
7	A-4	44.3	11.1	42.7	1.66	0.07	0.14	Fab1	81.75	0.0719	97	GFP	1	**
8	A-6	44.3	11.1	42.7	1.66	0.07	0.14	Fab1	96.83	0.0191	98	GFP	1	**
9	A-31	44.3	11.1	42.7	1.66	0.07	0.14	Fab1	103.2	0.0218	98	GFP	1	**
10	B-9	44.3	11.1	42.7	1.66	0.07	0.14	Fab1	79.95	0.0395	59	GFP	1	*
11	B-13	44.3	11.1	42.7	1.66	0.07	0.14	Fab1	130.4	0.0249	55	GFP	1	*
12	C-15	44.3	11.1	42.7	1.66	0.07	0.14	Fab1	82.44	0.1977	99	GFP	1	*
14	C-40	44.3	11.1	42.7	1.66	0.07	0.14	Fab1	123.6	0.2266	99	GFP	1	*
18	A-4	33.0	39.9	25.4	1.54	0.05	0.11	Fab1	93.4	0.089	87	GFP	1	**
19	A-6	33.0	39.9	25.4	1.54	0.05	0.11	Fab1	92.91	0.089	95	GFP	1	****
20	A-31	33.0	39.9	25.4	1.54	0.05	0.11	Fab1	87.62	0.082	97	GFP	1	**
21	B-9	33.0	39.9	25.4	1.54	0.05	0.11	Fab1	104.9	0.1272	83	GFP	1	*

22	DLinKDMA	33.0	39.9	25.4	1.54	0.05	0.11	Fab1	117.7	0.008	81	GFP	1	*
23	A-6	33.5	40.5	25.8	0.00	0.05	0.11	Fab1	103.3	0.112	95	GFP	0.075	***
24	A-6	24.7	45.9	29.2	0.00	0.06	0.12	Fab1	107.5	0.094	91	GFP	0.075	**
25	A-6	40.5	36.3	23.1	0.00	0.05	0.10	Fab1	106.9	0.113	97	GFP	0.075	****
26	A-6	39.1	30.7	30.1	0.00	0.06	0.13	Fab1	91.08	0.108	98	GFP	0.075	***
27	A-6	29.4	47.9	22.6	0.00	0.05	0.09	Fab1	128.6	0.157	91	GFP	0.075	***
28	A-6	36.8	44.5	18.4	0.00	0.06	0.12	Fab1	127.4	0.115	93	GFP	0.075	***
29	A-6	30.7	37.2	32.0	0.00	0.05	0.10	Fab1	93.93	0.08	97	GFP	0.075	***
30	A-6	29.4	35.5	34.8	0.00	0.07	0.15	Fab1	87.61	0.143	96	GFP	0.075	**
31	A-6	21.3	53.4	25.2	0.00	0.05	0.10	Fab1	126.7	0.163	87	GFP	0.075	**
32	A-6	33.5	40.5	25.8	0.00	0.05	0.11	Fab1	91.48	0.136	97	GFP	0.075	***
33	A-6	27.5	51.1	21.2	0.00	0.07	0.14	Fab1	95.19	0.0631	96	GFP	0.075	**
34	A-6	22.4	41.6	35.8	0.00	0.06	0.11	Fab1	82.25	0.119	98	GFP	0.075	**
35	A-6	46.4	27.0	26.5	0.00	0.06	0.11	Fab1	97.4	0.099	98	GFP	0.075	**
36	A-6	35.9	43.4	20.5	0.00	0.04	0.09	Fab1	105.5	0.116	97	GFP	0.075	***
37	A-6	44.1	39.4	16.3	0.00	0.05	0.10	Fab1	105.3	0.117	97	GFP	0.075	**
38	A-6	37.5	33.5	28.9	0.00	0.04	0.09	Fab1	91.85	0.0845	98	GFP	0.075	**
39	A-6	43.7	34.3	21.8	0.00	0.07	0.14	Fab1	93.1	0.079	98	GFP	0.075	**
40	A-6	35.3	27.8	36.7	0.00	0.06	0.11	Fab1	86.53	0.079	98	GFP	0.075	**
41	A-6	31.9	52.0	16.0	0.00	0.05	0.10	Fab1	114.4	0.117	95	GFP	0.075	**
42	A-6	33.5	40.5	25.8	0.00	0.05	0.11	Fab1	102.5	0.175	98	GFP	0.075	****
43	A-6	27.2	44.4	28.3	0.00	0.04	0.09	Fab1	116.8	0.178	97	GFP	0.075	***
44	A-6	33.5	40.5	25.8	0.00	0.08	0.17	Fab1	95.1	0.124	97	GFP	0.075	***
45	A-6	23.3	58.5	18.0	0.00	0.06	0.12	Fab1	162.9	0.176	85	GFP	0.075	*
46	A-6	26.2	31.7	41.9	0.00	0.06	0.13	Fab1	73.03	0.083	98	GFP	0.075	**
47	A-6	19.5	49.1	31.3	0.00	0.05	0.10	Fab1	133.5	0.215	95	GFP	0.075	***
48	A-6	51.1	29.7	19.0	0.00	0.06	0.12	Fab1	106.5	0.056	99	GFP	0.075	***
49	A-6	38.7	46.8	14.4	0.00	0.05	0.09	Fab1	143.1	0.197	94	GFP	0.075	***

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50	A-6	42.5	24.7	32.7	0.00	0.05	0.10	Fab1	105.8	0.051	99	GFP	0.075	***
51	A-6	33.5	40.5	25.8	0.00	0.04	0.08	Fab1	106.7	0.155	98	GFP	0.075	***
52	A-6	33.5	40.5	25.8	0.00	0.05	0.11	Fab2	120	0.088	88	GFP	0.1	***
53	A-17	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	98.71	0.074	73	GFP	1	****
54	A-17	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	98.71	0.074	73	GFP	0.3	****
55	A-17	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	98.71	0.074	73	GFP	0.1	****
56	A-17	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	98.71	0.074	73	GFP	0.03	***
57	A-6	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	75.36	0.12	89	GFP	1	***
58	A-6	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	75.36	0.12	89	GFP	0.3	***
59	A-6	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	75.36	0.12	89	GFP	0.1	**
60	A-6	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	75.36	0.12	89	GFP	0.03	*
61	A-6	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	86.87	0.142 4	88	GFP	0.1	****
62	A-32	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	122.1	0.050 8	84	GFP	1	****
63	A-32	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	122.1	0.050 8	84	GFP	0.3	****
64	A-32	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	122.1	0.050 8	84	GFP	0.1	****
65	A-32	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	122.1	0.050 8	84	GFP	0.03	**
66	A-33	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	104.9	0.086 5	89	GFP	1	****
67	A-33	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	104.9	0.086 5	89	GFP	0.3	****
68	A-33	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	104.9	0.086 5	89	GFP	0.1	***
69	A-33	33.0	39.9	25.4	1.54	0.05	0.11	Fab2	104.9	0.086 5	89	GFP	0.03	**
70	A-6	33.5	40.5	25.8	0.00	0.05	0.11	Fab2	92.4	0.078	88	GFP	0.05	***
71	A-6	33.5	40.5	25.8	0.00	0.05	0.11	Fab2	107	0.1	89	GFP	0.075	**
72	A-6	33.5	40.5	25.8	0.00	0.05	0.11	Fab3	87.5	0.04	83	GFP	0.075	***
73	ALC-315	33.1	40	25.5	1.5	0	0	Fab1	124.9	0.185	77	GFP	1	*

74	ALC-315	32.9	39.7	25.3	1.5	0.2	0.4	Fab1	134.3	0.109	83	GFP	1	***
75	ALC-315	33	39.9	25.4	1.5	0.1	0.1	Fab1	128.2	0.082	85	GFP	1	***

Table XI. Additional exemplary compositions described herein and properties thereof

Composition	Ionizable Lipid	IL mole %	DSPC mole %	Chol mole %	DMG-PEG2000 mole %	DSPE-PEG2000-Fab mole %	Fab source	Particle Size	PDI	EE	mRNA	Dose (mg/kg)	% GFP + T cells
76	A-6	34.0	32.25	32.25	1.45	0.05	Fab3	58.8	0.15	90	eGFP	0.1	**
77	A-40	34.0	32.25	32.25	1.45	0.05	Fab3	61.6	0.18	89	eGFP	0.1	*
78	A-41	34.0	32.25	32.25	1.45	0.05	Fab3	53.5	0.09	91	eGFP	0.1	*
79	A-6	33.0	40.0	25.4	1.55	0.05	Fab3	64.8	0.18	91	eGFP	0.1	***
80	A-6	33.0	40.0	25.4	1.55	0.05	Fab3	56.1	0.055	98	eGFP	0.1	**
81	A-6	33.0	40.0	25.4	1.55	0.05	Fab3	56.1	0.055	98	eGFP	0.03	
82	A-6	33.0	40.0	25.4	1.55	0.05(PEG1000)	Fab3	54	0.082	98	eGFP	0.1	**
83	A-6	33.0	40.0	25.4	1.55	0.05(PEG1000)	Fab3	54	0.082	98	eGFP	0.03	
84	A-6	33.0	40.0	25.4	1.55	0.05(PEG3400)	Fab3	55.9	0.045	98	eGFP	0.1	**
85	A-6	33.0	40.0	25.4	1.55	0.05(PEG3400)	Fab3	55.9	0.045	98	eGFP	0.03	*
86	A-6	33.0	40.0	25.4	1.55	0.05(PEG5000)	Fab3	55.2	0.050	98	eGFP	0.1	**
87	A-6	33.0	40.0	25.4	1.55	0.05(PEG5000)	Fab3	55.2	0.050	98	eGFP	0.03	*
88	A-4	33.0	40.0	25.4	1.55	0.05	Fab3	64.9	0.13	89	eGFP	0.1	****

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89	A-4	33.0	40.0	25.4	1.55	0.05	Fab3	64.9	0.13	89	eGFP	0.03	**
90	A-6	33.0	40.0	25.4	1.55	0.05	Fab3	82.0	0.09	88	eGFP	0.1	***
91	A-6	33.0	40.0	25.4	1.55	0.05	Fab3	82.0	0.09	88	eGFP	0.03	**
92	A-6	33.0	40.0	25.4	1.55	0.05	Fab3	62.6	0.056	96	eGFP	0.1	****
93	A-6	33.0	40.0	25.4	1.55	0.05	Fab3	62.6	0.056	96	eGFP	0.03	*
94	A-6	33.0	40.0	25.4	1.55	0.05	Fab3	63.71	0.10	89	eGFP	0.1	***
95	A-6	33.0	40.0	25.4	1.55	0.05	Fab3	64.87	0.08	82	eGFP	0.1	***
96	A-6	47.22	26.22	26.22	0.31	0.03	Fab3	84.45	0.07	95	eGFP	0.1	****
97	A-6	46.4	25.8	25.8	1.97	0.03	Fab3	72.89	0.10	94	eGFP	0.1	***
98	A-6	46.3	28.9	24.4	0.4	0.03	Fab3	74.55	0.05	96	eGFP	0.1	****
99	A-6	46.3	28.9	24.4	0.4	0.03	Fab3	74.55	0.05	96	eGFP	0.03	**
100	A-6	43.7	26.2	29.3	0.7	0.03	Fab3	70.15	0.06	96	eGFP	0.1	****
101	A-6	43.7	26.2	29.3	0.7	0.03	Fab3	70.15	0.06	96	eGFP	0.03	**
102	A-6	41.7	22.8	34.6	0.9	0.04	Fab3	71.46	0.06	94	eGFP	0.1	***
103	A-6	41.7	22.8	34.6	0.9	0.04	Fab3	71.46	0.06	94	eGFP	0.03	*
104	A-6	48.1	33.6	17.1	1.2	0.04	Fab3	72.64	0.06	96	eGFP	0.1	****
105	A-6	48.1	33.6	17.1	1.2	0.04	Fab3	72.64	0.06	96	eGFP	0.03	***
106	A-6	48.6	34.6	16.4	0.4	0.04	Fab3	86.0	0.03	97	eGFP	0.1	****
107	A-6	48.6	34.6	16.4	0.4	0.04	Fab3	86.0	0.03	97	eGFP	0.03	**
108	A-6	33.0	40.0	25.4	1.55	0.05	Fab3	58.89	0.07	91	eGFP	0.1	***
109	A-6	48.57	34.59	16.43	0.36	0.04	Fab3	84.09	0.06	94	eGFP	0.1	****
110	A-6	35.6	16.35	47.42	0.59	0.06	Fab3	65.3	0.01	93	eGFP	0.1	
111	A-6	47.11	16.84	35.14	0.87	0.04	Fab3	75.9	0.02	93	eGFP	0.1	**
112	A-6	35.77	34.34	28.73	1.12	0.04	Fab3	76.28	0.11	91	eGFP	0.1	**

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113	A-6	37.39	30.49	29.91	2.13	0.09	Fab3	56.79	0.06	85	eGFP	0.1	*
114	A-6	39.64	30.12	29.33	0.87	0.05	Fab3	67.35	0.06	93	eGFP	0.1	***
115	A-6	36.03	34.49	27.75	1.68	0.06	Fab3	63.86	0.08	88	eGFP	0.1	*
116	A-6	36.58	35.02	28.17	0.18	0.06	Fab3	72.24	0.07	93	eGFP	0.1	**
117	A-6	42.05	34.56	22.02	1.33	0.05	Fab3	74.87	0.09	93	eGFP	0.1	***
118	A-6	42.56	34.97	22.28	0.14	0.05	Fab3	84.04	0.02	94	eGFP	0.1	***
119	A-6	33.04	39.93	25.44	1.54	0.05	Fab3	72.68	0.08	89	eGFP	0.1	**
120	A-4	33.5	40.5	25.8	0.20	0.05	Fab3	107.6	0.16	95	eGFP	1.0	****
121	A-4	33.5	40.5	25.8	0.20	0.05	Fab3	107.6	0.16	95	eGFP	0.1	**
122	A-4	33.5	40.5	25.8	0.20	0.05	Fab3	107.6	0.16	95	eGFP	0.03	*
123	A-4	44.4	11.2	42.7	1.7	0.07	Fab3	92.8	0.08	96	eGFP	1.0	**
124	A-4	44.4	11.2	42.7	1.7	0.07	Fab3	92.8	0.08	96	eGFP	0.1	*
125	A-4	44.4	11.2	42.7	1.7	0.07	Fab3	92.8	0.08	96	eGFP	0.03	
126	A-6	33.5	40.5	25.8	0.20	0.05	Fab3	91.2	0.16	96	eGFP	1.0	****
127	A-6	33.5	40.5	25.8	0.20	0.05	Fab3	91.2	0.16	96	eGFP	0.1	***
128	A-6	33.5	40.5	25.8	0.20	0.05	Fab3	91.2	0.16	96	eGFP	0.03	
129	A-6	44.4	11.2	42.7	1.7	0.07	Fab3	87.4	0.09	95	eGFP	1.0	**
130	A-6	44.4	11.2	42.7	1.7	0.07	Fab3	87.4	0.09	95	eGFP	0.1	*
131	A-6	44.4	11.2	42.7	1.7	0.07	Fab3	87.4	0.09	95	eGFP	0.03	
132	A-4	33.0	39.9	25.4	1.5	0.05	Fab3	105.48	0.16	86	eGFP	1.0	****
133	A-4	33.0	39.9	25.4	1.5	0.05	Fab3	105.48	0.16	86	eGFP	0.1	***
134	A-4	33.0	39.9	25.4	1.5	0.05	Fab3	105.48	0.16	86	eGFP	0.03	*
135	A-4	33.1	39.9	25.5	1.5	0.02	Fab3	110.4	0.13	88	eGFP	1.0	****
136	A-4	33.1	39.9	25.5	1.5	0.02	Fab3	110.4	0.13	88	eGFP	0.1	**
137	A-4	33.1	39.9	25.5	1.5	0.02	Fab3	110.4	0.13	88	eGFP	0.03	

138	A-6	33.0	39.9	25.4	1.5	0.05	Fab3	77.0	0.12	88	eGFP	1.0	****
139	A-6	33.0	39.9	25.4	1.5	0.05	Fab3	77.0	0.12	88	eGFP	0.1	***
140	A-6	33.0	39.9	25.4	1.5	0.05	Fab3	77.0	0.12	88	eGFP	0.03	*
141	A-6	33.1	39.9	25.5	1.5	0.02	Fab3	82.0	0.17	83	eGFP	1.0	****
142	A-6	33.1	39.9	25.5	1.5	0.02	Fab3	82.0	0.17	83	eGFP	0.1	**
143	A-6	33.1	39.9	25.5	1.5	0.02	Fab3	82.0	0.17	83	eGFP	0.03	*

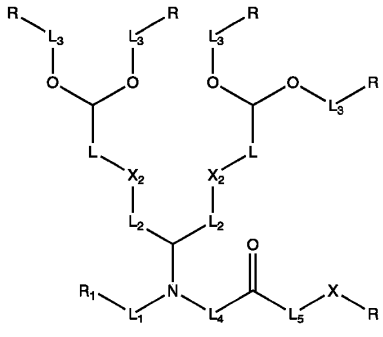
* >10%, ** 20-40%, *** 40-50%, ****>50%
Fab1 Digested mAb
Fab2 Cellularly produced Fab
Fab3 Cellularly produced Fab'

WO 2025/174825 PCT/US2025/015513

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference. Other embodiments are within the claims.

CLAIMS
What is claimed is:

1. A compound of formula (A-II):


(A-II)

or its N-oxide, isomer, or a pharmaceutically acceptable salt thereof, wherein

L is optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

L_1 is optionally substituted C_{1-6} alkylenyl, or C_{2-6} heteroalkylenyl;

each L_2 is independently C_{1-6} alkylenyl;

L_3 is optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

L_4 is absent, optionally substituted C_{1-10} alkylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

L_5 is optionally substituted C_{1-10} alkyl, optionally substituted C_{1-10} alkenyl, optionally substituted C_{1-10} alkynyl, or optionally substituted C_{2-10} heteroalkylenyl;

X is absent, -OC(O)-, -C(O)O-, -NHC(O)-, -C(O)NH-, or -OC(O)O-;

each X_2 is absent, -OC(O)-, -C(O)O-, -NHC(O)-, -C(O)NH-, or -OC(O)O-;

each R is independently hydrogen, OR_6, or an optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, or phenyl;

R_1 is hydrogen, optionally substituted phenyl, optionally substituted 3- to 7-membered cycloaliphatic, optionally substituted 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 8- to 10-membered bicyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, $-OR_2$, $-C(O)OR_2$, $-C(O)SR_2$, $-OC(O)R_2$, $-OC(O)OR_2$, -CN, $-N(R_2)_2$, $-C(O)N(R_2)_2$, $-S(O)_2N(R_2)_2$, $-NR_2C(O)R_2$, $-OC(O)N(R_2)_2$, $-N(R_2)C(O)OR_2$, $-NR_2S(O)_2R_2$, $-NR_2C(O)N(R_2)_2$, $-NR_2C(S)N(R_2)_2$, $-NR_2C(NR_2)N(R_2)_2$, $-NR_2C(CHR_2)N(R_2)_2$, $-N(OR_2)C(O)R_2$, $-N(OR_2)S(O)_2R_2$, $-N(OR_2)C(O)OR_2$, $-N(OR_2)C(O)N(R_2)_2$, $-N(OR_2)C(S)N(R_2)_2$, $-N(OR_2)C(NR_2)N(R_2)_2$, $-N(OR_2)C(CHR_2)N(R_2)_2$, $-C(NR_2)N(R_2)_2$, $-C(NR_2)R_2$, $-C(O)N(R_2)OR_2$, $-C(R_2)N(R_2)_2C(O)OR_2$, $-CR_2(R_3)_2$, $-OP(O)(OR_2)_2$, or $-P(O)(OR_2)_2$; or

R_1 is a ring selected from 3- to 7-membered cycloaliphatic and 3- to 7- membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein the cycloaliphatic or heterocyclyl ring is optionally substituted with 1-4 R_2 or R_3 groups;

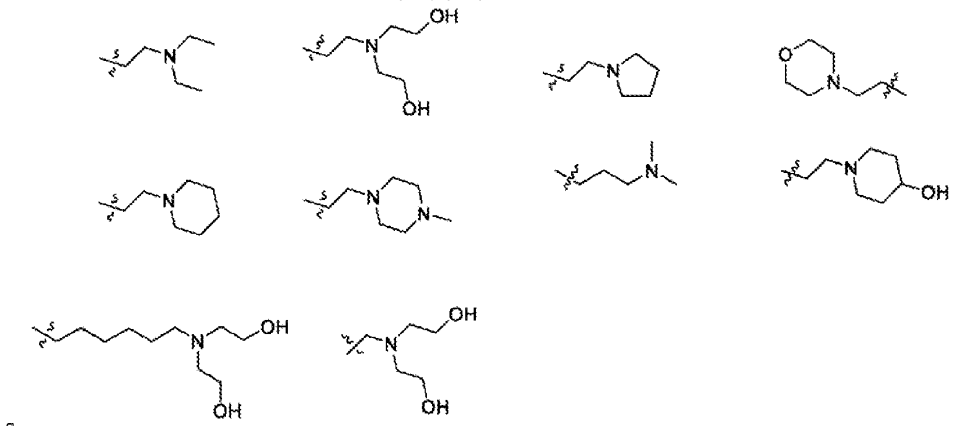
each R_2 is independently hydrogen, oxo, -CN, $-NO_2$, $-OR_4$, $-S(O)_2R_4$, $-S(O)_2N(R_4)_2$, $-(CH_2)_n-R_4$, or an optionally substituted group selected from C_{1-6} aliphatic, phenyl, 3- to 7-membered cycloaliphatic, 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, and 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or two occurrences of R_2, taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from

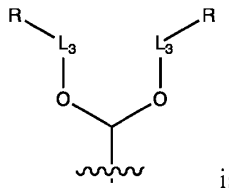
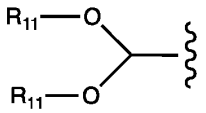
nitrogen, oxygen, or sulfur; each R_3 is independently $-(CH_2)n-R_4$; or two occurrences of R_3, taken together with the atom(s) to which they are attached, form optionally substituted 5- to 6-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, or sulfur; each R_4 is independently hydrogen, $-OR_5$, $-N(R_5)_2$, $-OC(O)R_5$, $-OC(O)OR_5$, $-CN$, $-C(O)N(R_5)_2$, $-NR_5C(O)R_5$, $-OC(O)N(R_5)_2$, $-N(R_5)C(O)OR_5$, $-NR_5S(O)_2R_5$, $-NR_5C(O)N(R_5)_2$, $-NR_5C(S)N(R_5)_2$, or $-NR_5C(NR_5)N(R_5)_2$; and

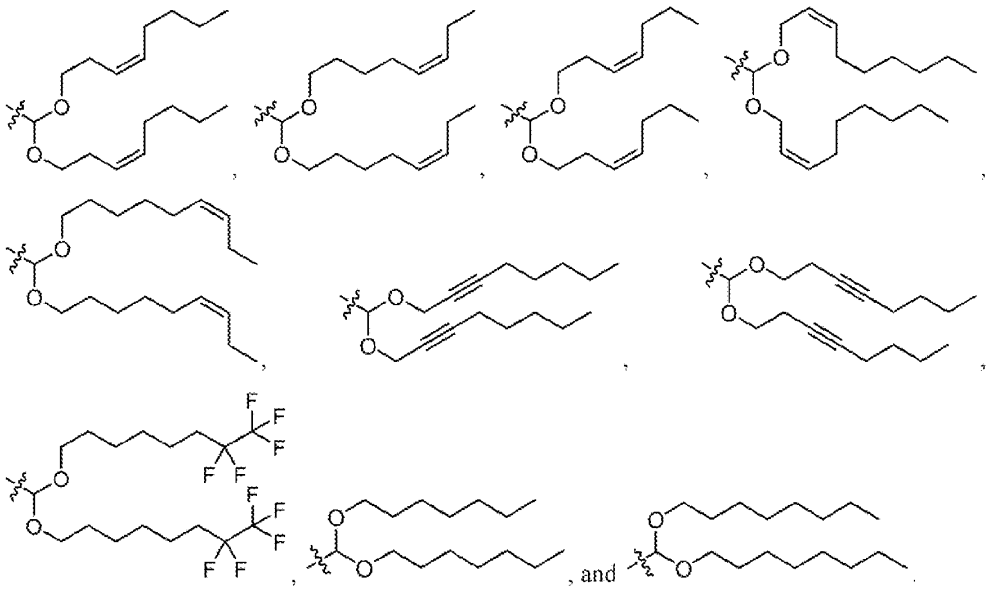
each R_5 is independently hydrogen, or optionally substituted C_{1-6} aliphatic; or two occurrences of R_5, taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur; each R_6 is independently C_{4-12} aliphatic; and each n is independently 0 to 4.

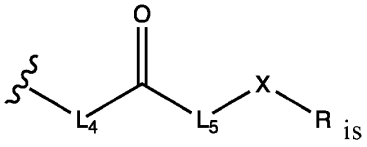
2. The compound for claim 1, wherein R_1 is $-N(R_2)_2$.

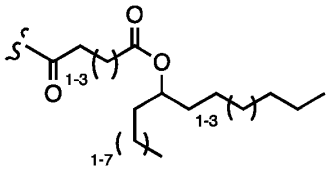
3. The compound of claim 1, wherein L_1-R_1 is selected from the group consisting of

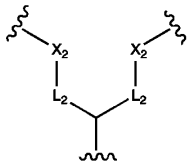
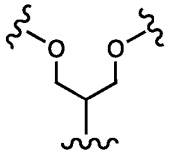


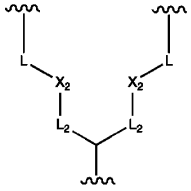
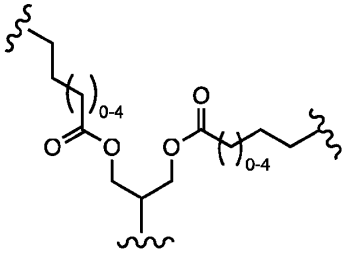
4. The compound of claim 1, wherein,  is selected from the group consisting of: 



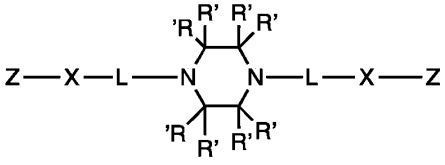
5. The compound of claim 1, wherein  is



6. The compound of claim 1, wherein  is .

7. The compound of claim 1, wherein  is .

8. A compound of formula (B-I):



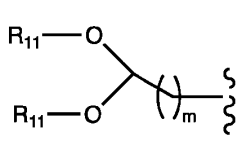
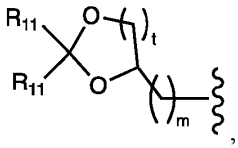
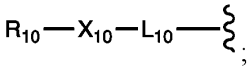
(B-I)

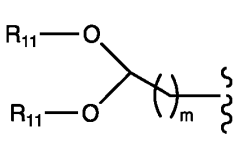
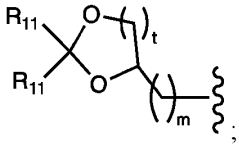
or its N-oxide, isomer, or a pharmaceutically acceptable salt thereof, wherein

each R' is independently absent, optionally substituted C_{1-6} alkyl, or optionally substituted C_{1-6} heteroalkyl;

each L is independently optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

each X is independently O, S, N(R"), S-S, OC(O), C(O)O, C(O)NH, NHC(O), OC(O)O, OC(O)NH, or NHC(O)O;

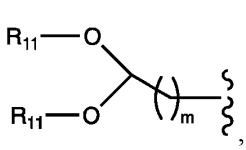
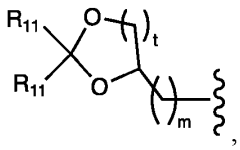
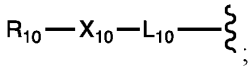
each Z is independently , , or ;

provided that at least one or two of Z is  or ;

each of m is independently 1-9;

t is 1, 2 or 3;

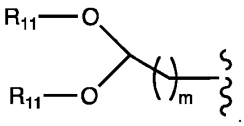
X_{10} is absent, O, S, N(R"), S-S, OC(O), C(O)O, C(O)NH, NHC(O), OC(O)O, OC(O)NH, or NHC(O)O;

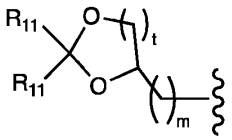
R" is H, , , or ;

L_{10} is optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

each R_{11} is independently C_{2-20} aliphatic, 3- to 12-membered cycloaliphatic, C_2-C_{10} aliphatic substituted with 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl; and

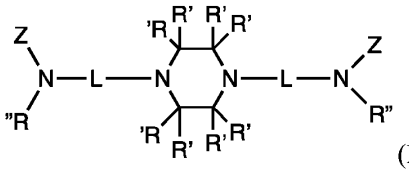
each R_{10} is independently hydrogen, OR_6, or an optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl.

9. The compound of claim 8, wherein Z is .

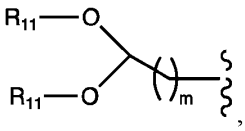
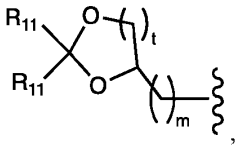
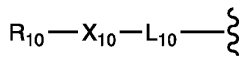
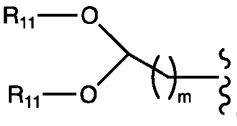
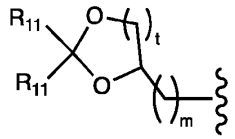
10. The compound of claim 8, wherein Z is .

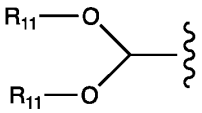
11. The compound of claim 8, where R' is H or CH_3.

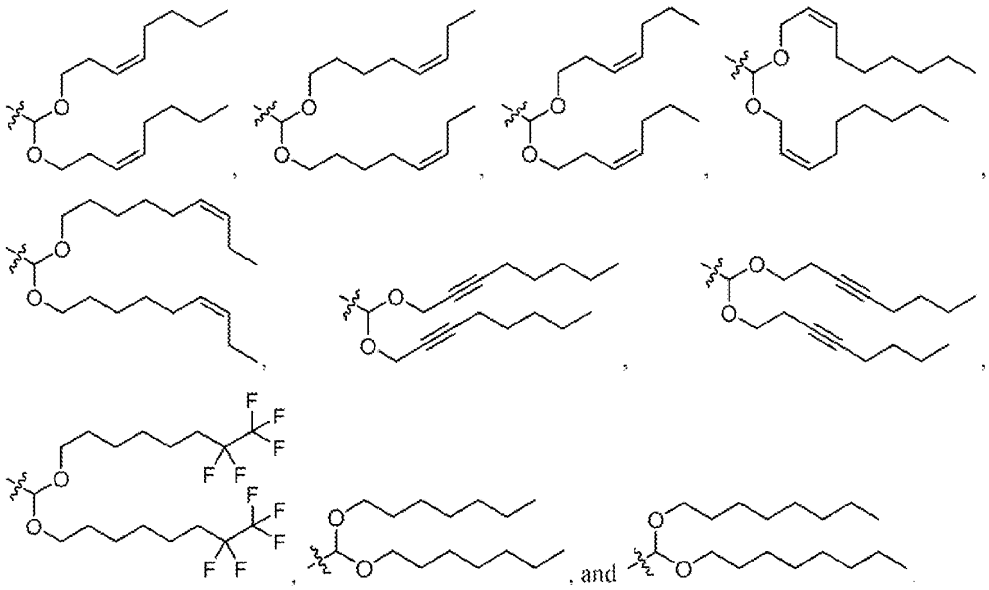
12. The compound of claim 8 represented by formula (B-II):

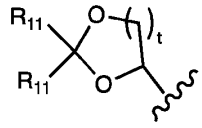


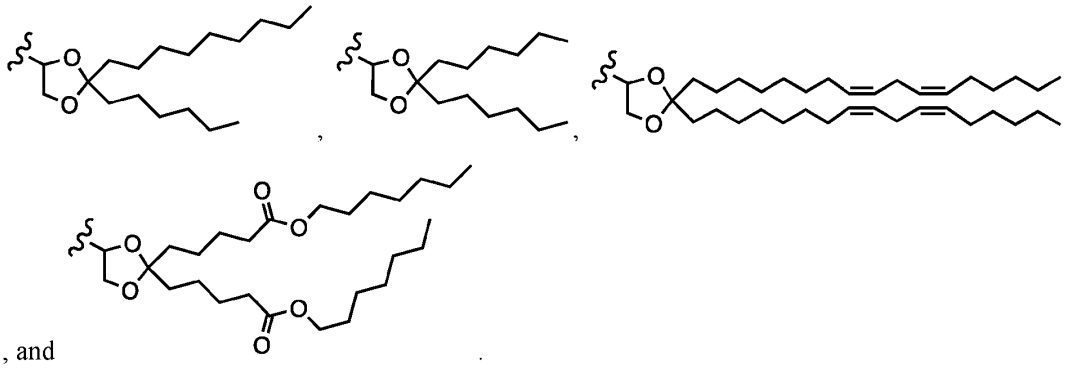
(B-II),

wherein Z and R" are each independently , , or ; provided at least two or three of Z and R" are  or .

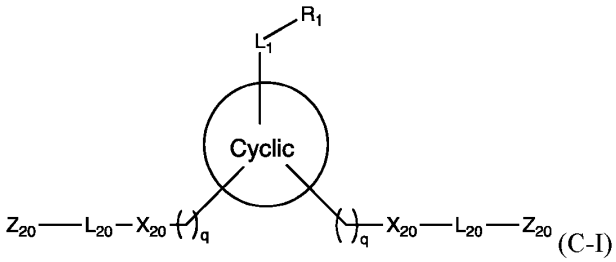
13. The compound of claims 8-12, wherein  is selected from the group consisting of:



14. The compound of claims 8-12, wherein  is selected from the group consisting of:



15. A compound of formula (C-I):

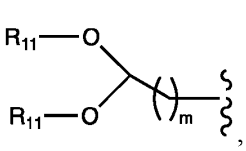
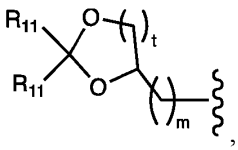
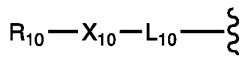
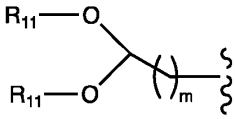
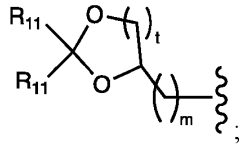


(C-I)

or its N-oxide, isomer, or a pharmaceutically acceptable salt thereof, wherein

each of X_{20} is independently absent, O, S, N(R"), S-S, OC(O), C(O)O, C(O)NH, NHC(O), OC(O)O, OC(O)NH, or NHC(O)O;

each of L_{20} is independently optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

each of Z_{20} is independently , , or ; provided that at least one of Z_{20} is  or ;

each of m is 1-9;

t is 1, 2 or 3;

L_{10} is optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

each of R_{11} is independently C_{2-20} aliphatic, 3- to 12-membered cycloaliphatic, C_2-C_{10} aliphatic substituted with 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, or phenyl; and

each of R_{10} is independently hydrogen, OR_6, or an optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, or phenyl;

q is 0, 1, 2, 3, 4, 5, 6, 7, 8, or 9.



is 4- to 8- membered cyclic or heterocyclic ring;

L_1 is absent, C_{1-6} alkylenyl, or C_{2-6} heteroalkylenyl;

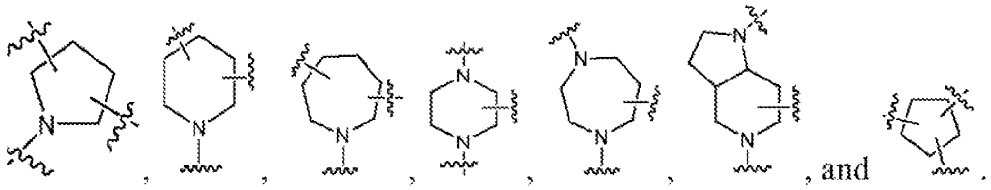
R_1 is hydrogen, optionally substituted phenyl, optionally substituted 3- to 7-membered cycloaliphatic, optionally substituted 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, optionally substituted 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, optionally substituted 8- to 10-membered bicyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur,
$-OR_2$, $-C(O)OR_2$, $-C(O)SR_2$, $-OC(O)R_2$, $-OC(O)OR_2$, $-CN$, $-N(R_2)_2$, $-C(O)N(R_2)_2$, $-S(O)_2N(R_2)_2$, $-NR_2C(O)R_2$, $-OC(O)N(R_2)_2$, $-N(R_2)C(O)OR_2$, $-NR_2S(O)_2R_2$, $-NR_2C(O)N(R_2)_2$, $-NR_2C(S)N(R_2)_2$, $-NR_2C(NR_2)N(R_2)_2$, $-NR_2C(CHR_2)N(R_2)_2$, $-N(OR_2)C(O)R_2$, $-N(OR_2)S(O)_2R_2$, $-N(OR_2)C(O)OR_2$, $-N(OR_2)C(O)N(R_2)_2$, $-N(OR_2)C(S)N(R_2)_2$, $-N(OR_2)C(NR_2)N(R_2)_2$, $-N(OR_2)C(CHR_2)N(R_2)_2$, $-C(NR_2)N(R_2)_2$, $-C(NR_2)R_2$, $-C(O)N(R_2)OR_2$, $-C(R_2)N(R_2)_2C(O)OR_2$, $-CR_2(R_3)_2$, $-OP(O)(OR_2)_2$, or $-P(O)(OR_2)_2$; or
R_1 is a ring selected from 3- to 7-membered cycloaliphatic and 3- to 7- membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein the cycloaliphatic or heterocyclyl ring is optionally substituted with 1-4 R_2 or R_3 groups;

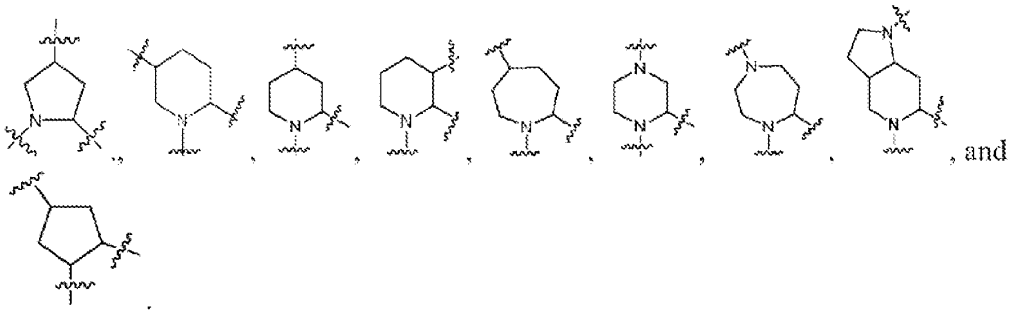
each R_2 is independently hydrogen, oxo, -CN, -NO_2, -OR_4, -$S(O)_2R_4$, -$S(O)_2N(R_4)_2$, -(CH_2)n-R_4, or an optionally substituted group selected from C_{1-6} aliphatic, phenyl, 3- to 7-membered cycloaliphatic, 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, and 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or two occurrences of R_2, taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur; each R_3 is independently -$(CH_2)_n$-R_4; or two occurrences of R_3, taken together with the atom(s) to which they are attached, form optionally substituted 5- to 6-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, or sulfur; each R_4 is independently hydrogen, -OR_5, -$N(R_5)_2$, -$OC(O)R_5$, -$OC(O)OR_5$, -CN, -$C(O)N(R_5)_2$, -$NR_5C(O)R_5$, -$OC(O)N(R_5)_2$, -$N(R_5)C(O)OR_5$, -$NR_5S(O)_2R_5$, -$NR_5C(O)N(R_5)_2$, -$NR_5C(S)N(R_5)_2$, or -$NR_5C(NR_5)N(R_5)_2$; and

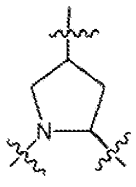
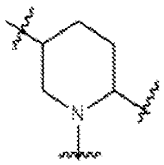
each R_5 is independently hydrogen, or optionally substituted C_{1-6} aliphatic; or two occurrences of R_5, taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, or sulfur; each R_6 is independently C_{4-12} aliphatic; and each n is independently 0 to 4.

16. The compound of claim 15, wherein the 

is selected from the group consisting of


N
N
N

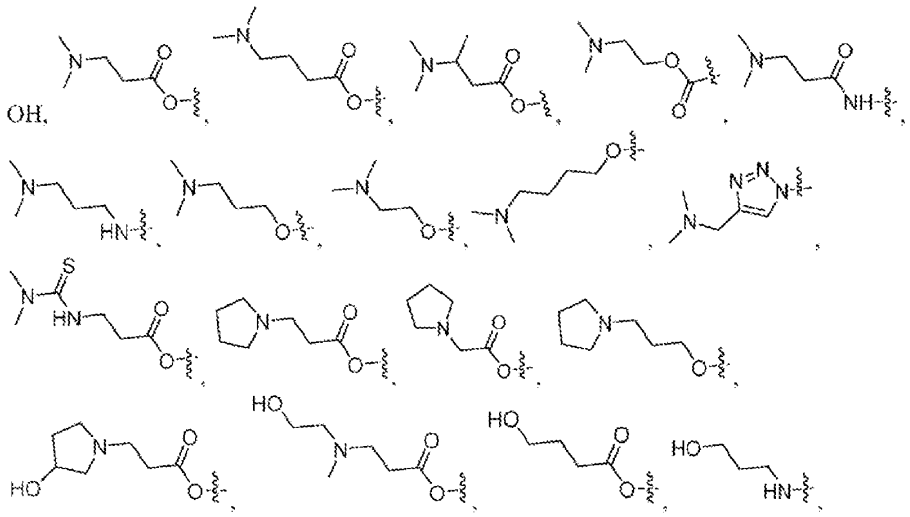


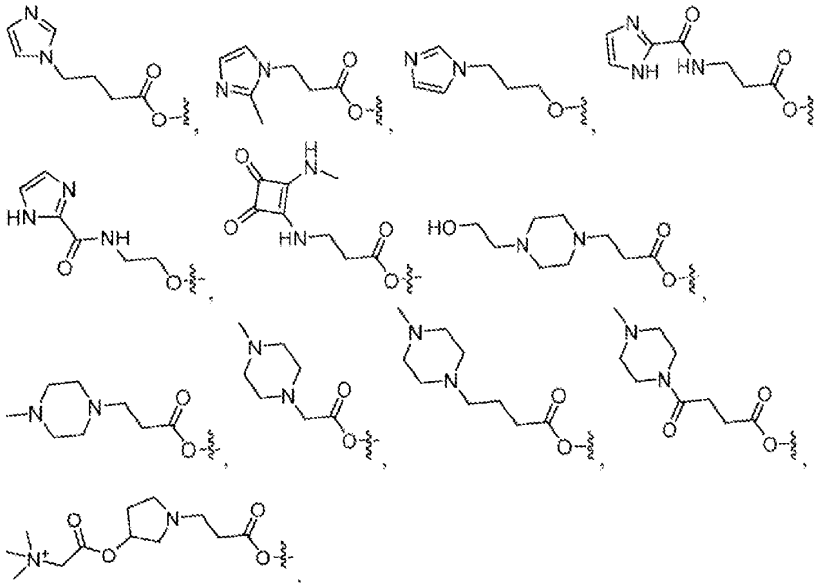
18. The compound of claim 15, wherein the Cyclic is  or .

19. The compound of claims 15-18, wherein L_1 is absent, and R_1 is $-OR_2$, $-C(O)OR_2$, $-C(O)SR_2$, $-OC(O)R_2$, $-OC(O)OR_2$, $-N(R_2)_2$, $-C(O)N(R_2)_2$, $-S(O)_2N(R_2)_2$, $-NR_2C(O)R_2$, $-OC(O)N(R_2)_2$, $-N(R_2)C(O)OR_2$, $-NR_2S(O)_2R_2$, or $-NR_2C(O)N(R_2)_2$; and R_2 is as defined in claim 15.

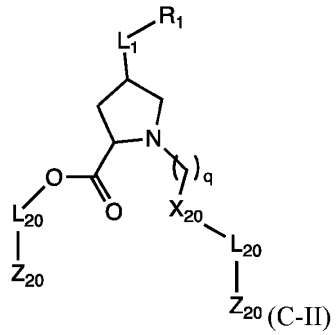
20. The compound of claim 15, wherein R_1 is $-OR_2$ or $-C(O)OR_2$.

21. The compound of claim 15, wherein L_1-R_1 is selected from the group consisting of



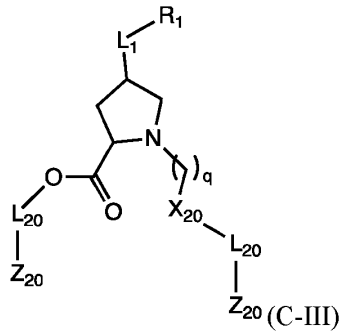

, and

22. The compound of claim 15 represented by formula (C-II):


(C-II)

wherein L_1 is absent, R_1 is OR_2, q is 2-9, X_{20} is OC(O) or C(O)O, R_2, L_{20} and Z_{20} are as defined in claim 15.

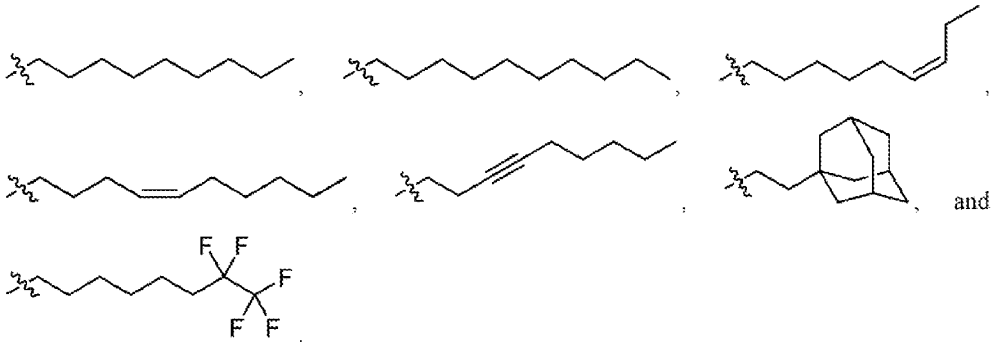
23. The compound of claim 15 represented by formula (C-III):



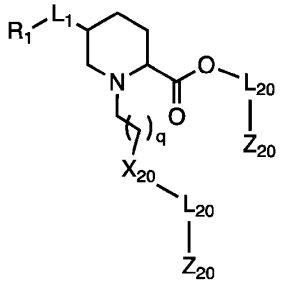
(C-III)

wherein L_1 is absent, R_1 is OR_2, q is 2-9, X_{20} is OC(O) or C(O)O, R_2, L_{20} and Z_{20} are as defined in claim 15.

24. The compound of claim 15, wherein $-L_{20}-Z_{20}$ is selected from the group consisting of:

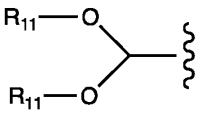

and

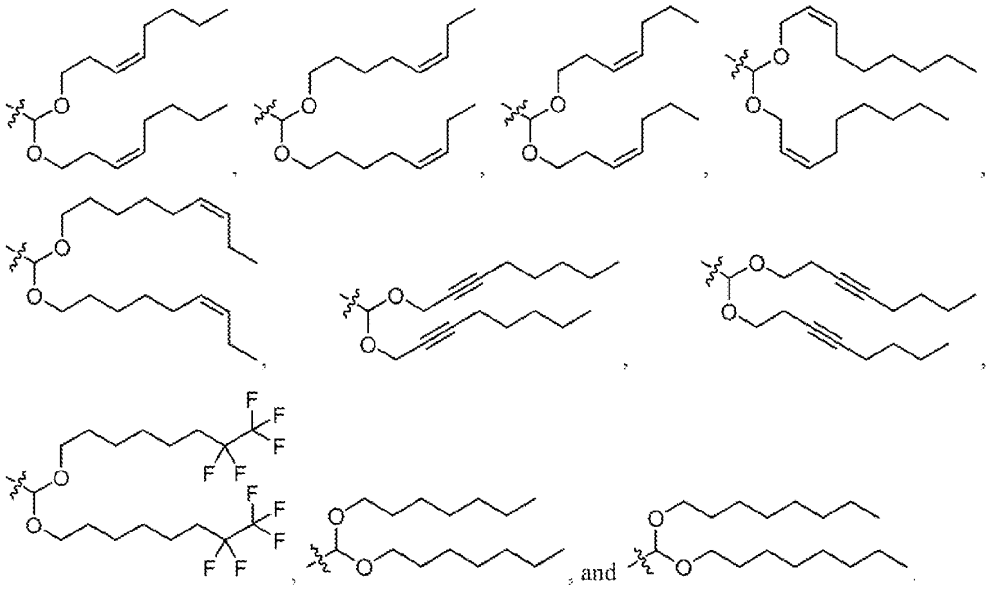
25. The compound of claim 15 represented by formula (C-IV):

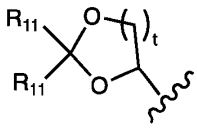


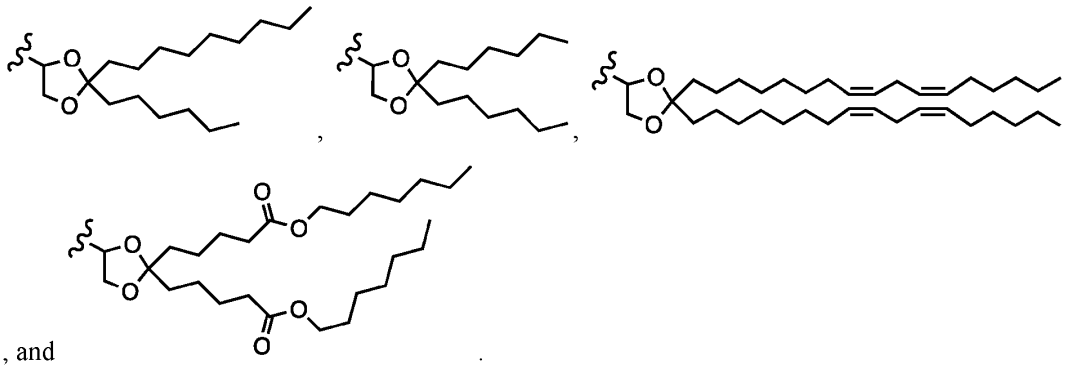
(C-IV)

wherein L_1 is absent, R_1 is OR_2, q is 2-9, X_{20} is OC(O) or C(O)O, R_2, L_{20} and Z_{20} are as defined in claim 15.

26. The compound of claims 15-25, where in  is selected from the group consisting of:

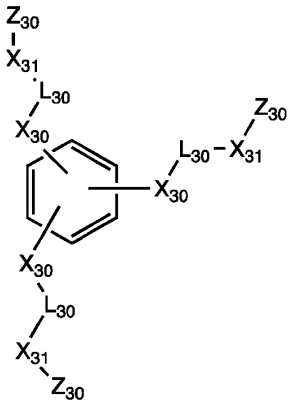


27. The compound of claims 15-25, wherein  is selected from the group consisting of:



, and .

28. A compound of formula (E-I):

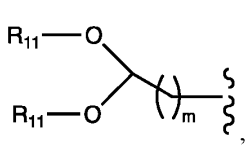
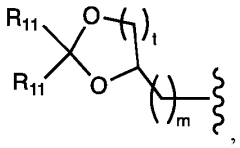
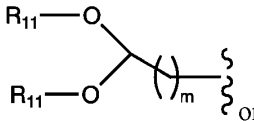
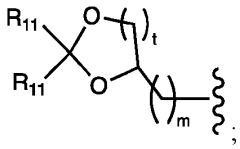


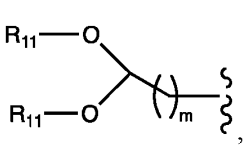
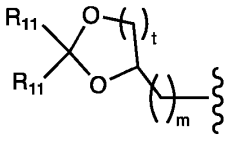
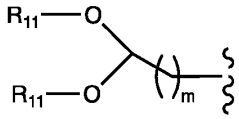
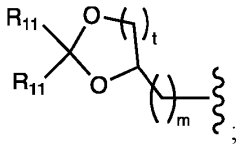
(E-I)

or its N-oxide, isomer, or a pharmaceutically acceptable salt thereof, wherein

each of X_{30} is independently absent, O, S, N(R"), S-S, OC(O), C(O)O, C(O)NH, NHC(O), OC(O)O, OC(O)NH, or NHC(O)O;

each of L_{30} is independently optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

each of X_{31} is independently NR"; wherein R" is H, , , or ; provided that at least one of Z_{30} is or ;

each of Z_{30} is independently , , or ; provided that at least one of Z_{30} is or ;

each of m is independently 1 to 9;

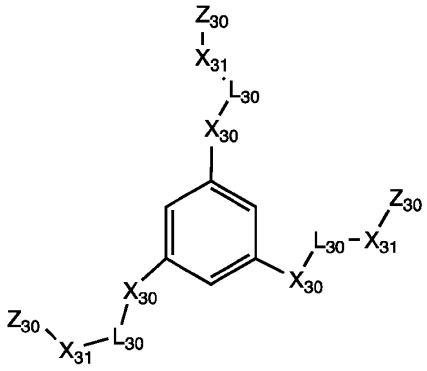
t is 1, 2 or 3;

L_{10} is optionally substituted C_{1-10} alkylenyl, optionally substituted C_{1-10} alkenylenyl, optionally substituted C_{1-10} alkynylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

Each of R_{11} is independently C_{2-20} aliphatic, 3- to 12-membered cycloaliphatic, C_2-C_{10} aliphatic substituted with 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, or phenyl; and

each of R_{10} is an independently optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, or phenyl.

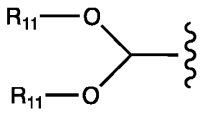
29. The compound of claim 28, represented by formula (E-II)

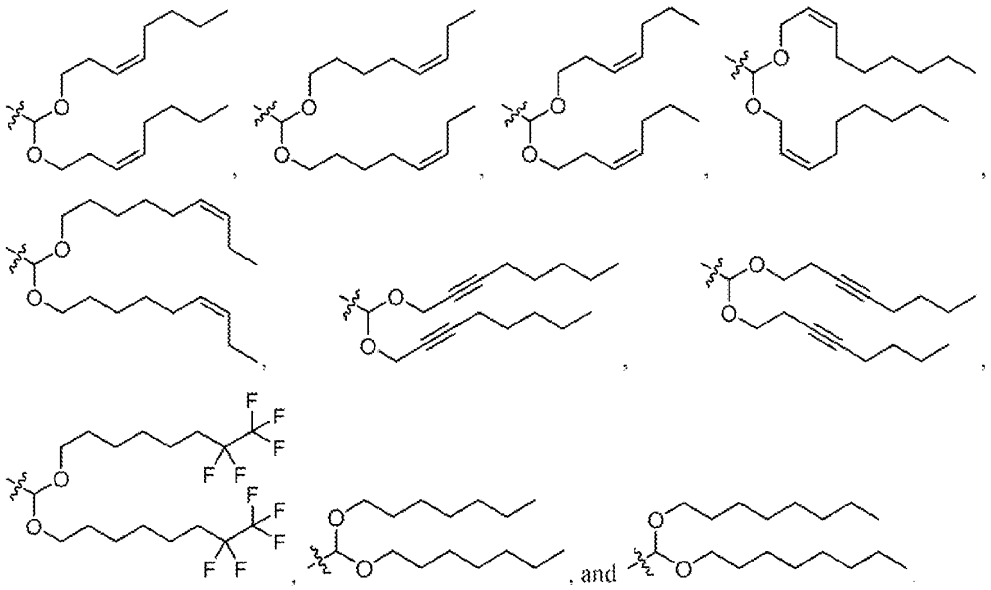


(E-II)

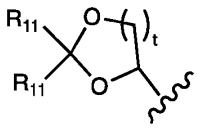
wherein X_{30}, L_{30}, X_{31} and Z_{30} are as defined in claim 20.

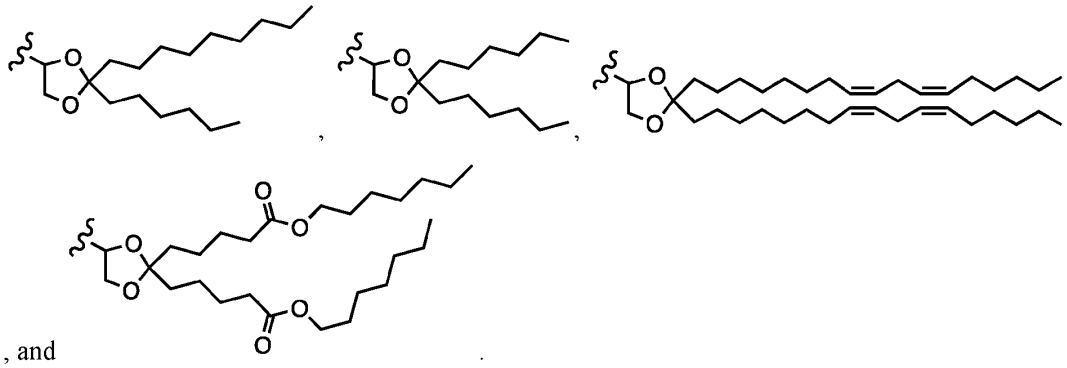
30. The compound of claim 28, wherein X_{30} is C(O)O, OC(O), C(O)NH, NHC(O) or O.

31. The compound of claims 28-30, where in  is selected from the group consisting of:



, and

32. The compound of claims 28-30, wherein  is selected from the group consisting of:



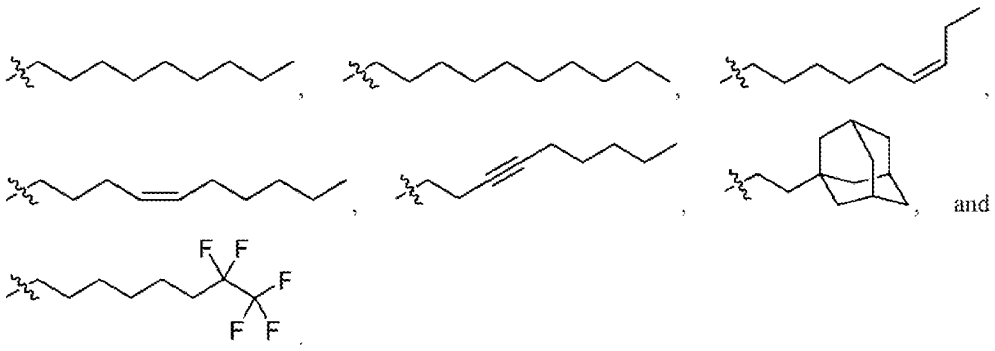
, and .

33. The compound of claim 28, wherein X_{30} is C(O)NH.

34. The compound of claim 33, wherein X_{30} is C(O)O.

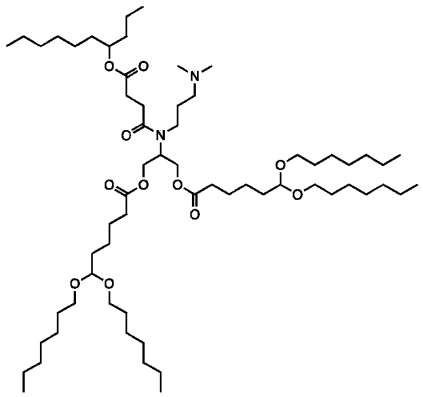
35. The compound of claim 28, wherein X_{31} is N-R".

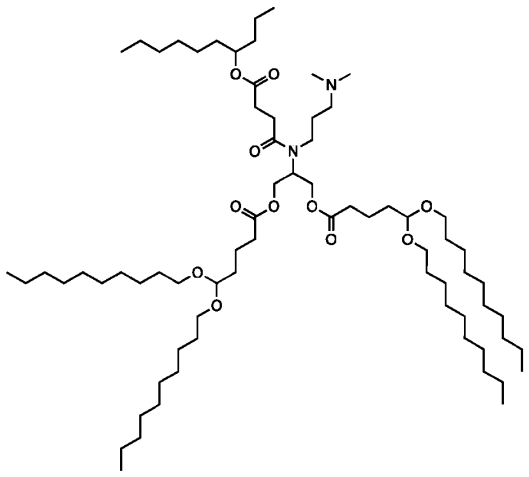
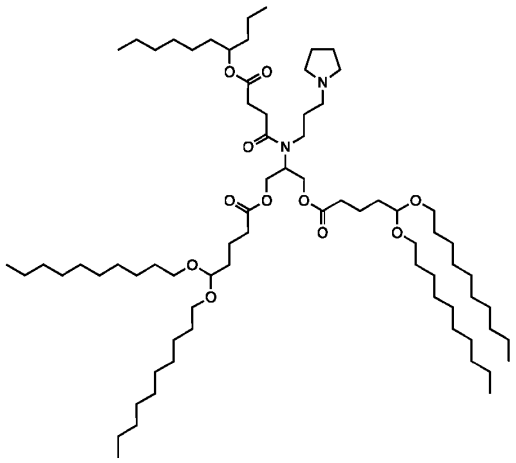
36. The compound of claim 28, wherein Z_{30} is selected from the group consisting of:

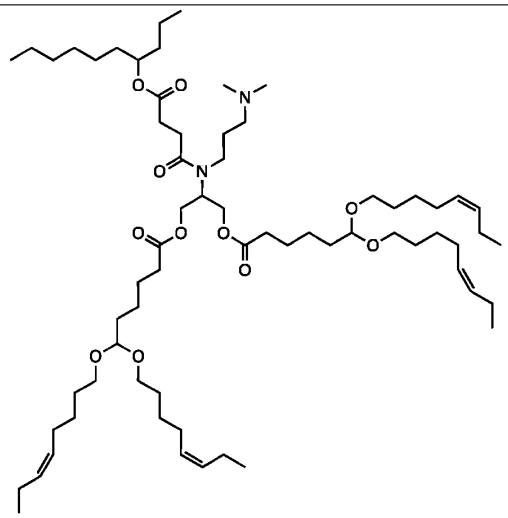
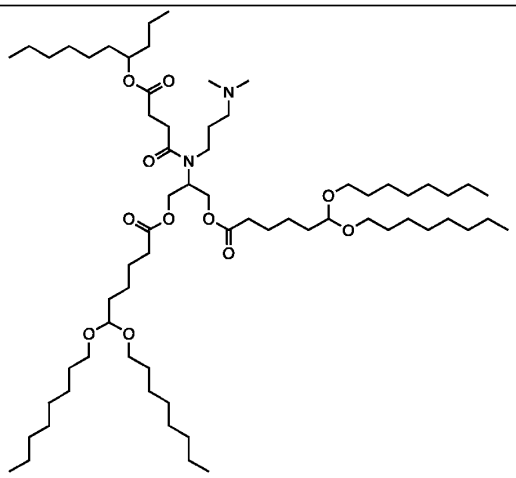

and

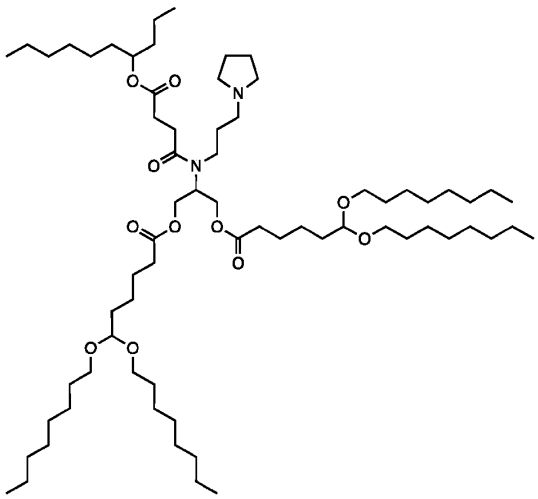
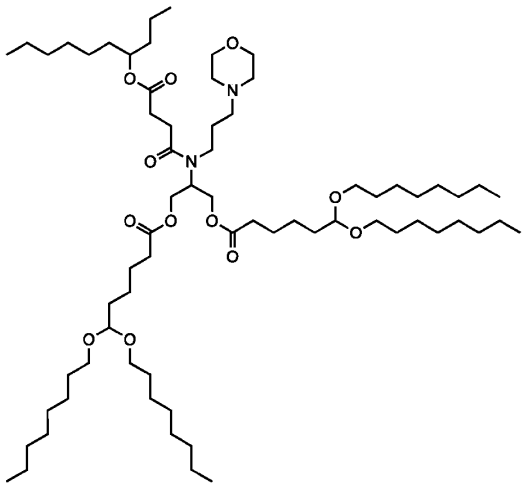
37. The compound of any one of claims 1-36, wherein the pKa of the protonated form of the compound is from about 4.5 to about 8.0.

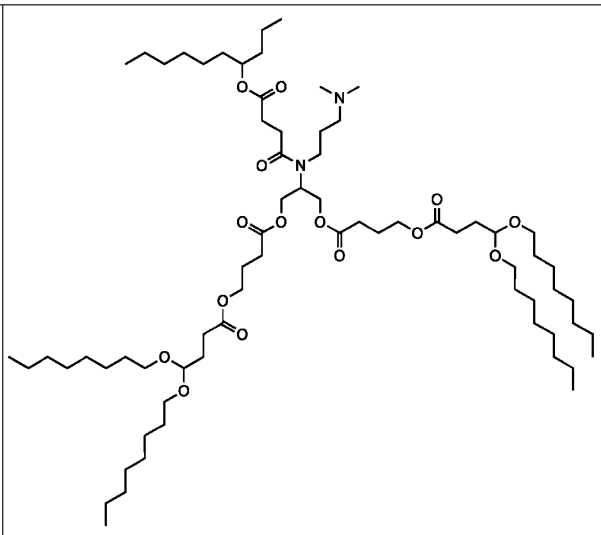
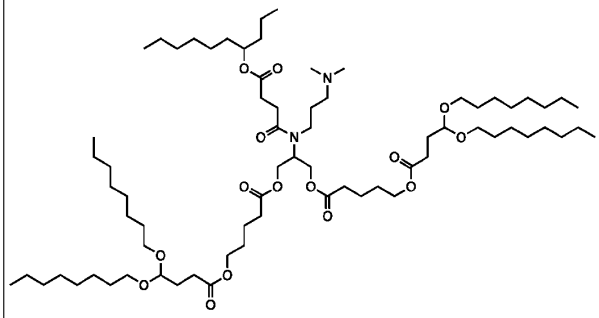
38. The compound of claim 1, having one of the following structures:

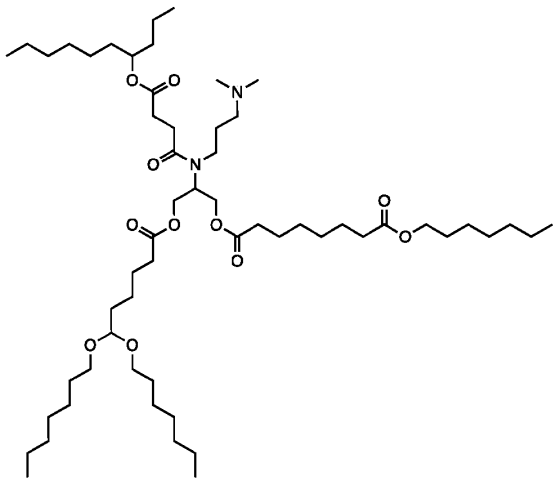
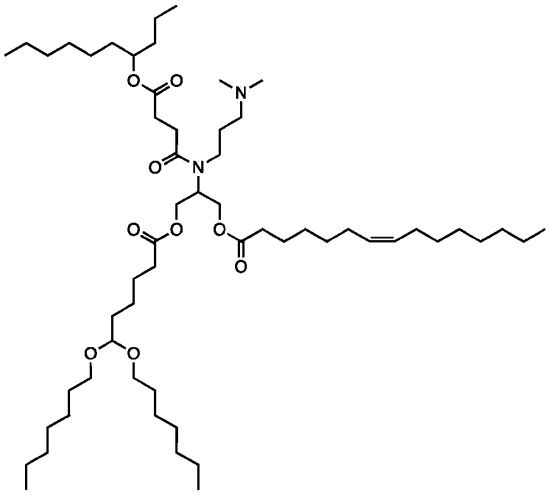
Compound	Structure	Name
A-1		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(heptyloxy)hexanoate)

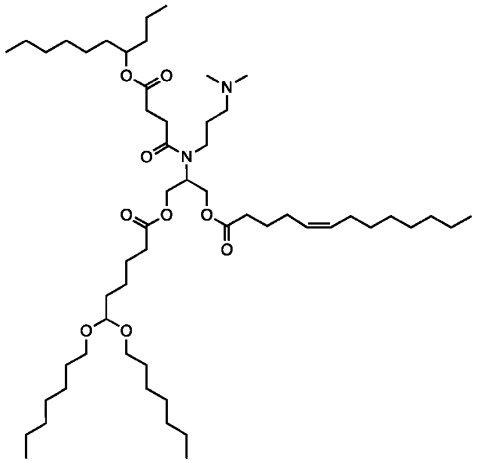
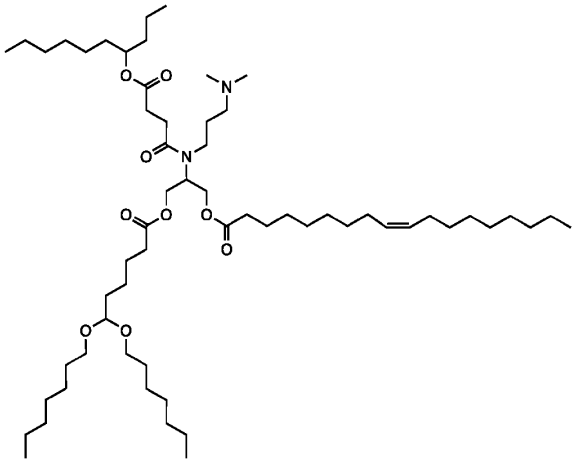
A-2		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-3		2-(4-(decan-4-yloxy)-4-oxo-N-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

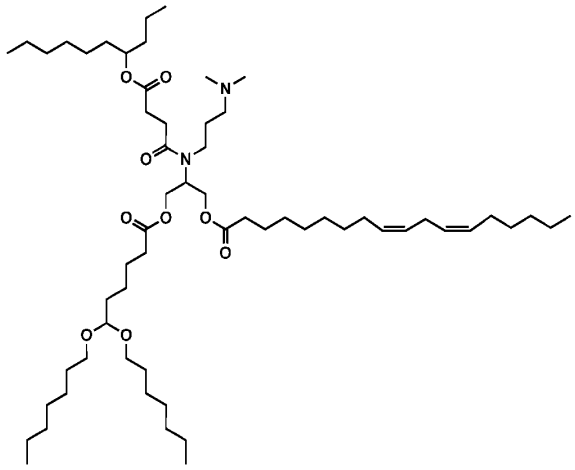
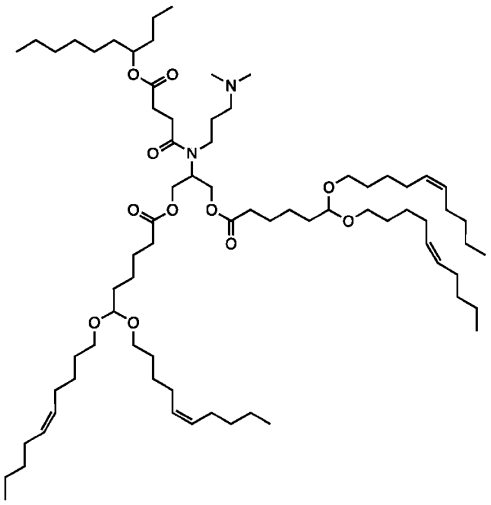
A-4		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)
A-5		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)

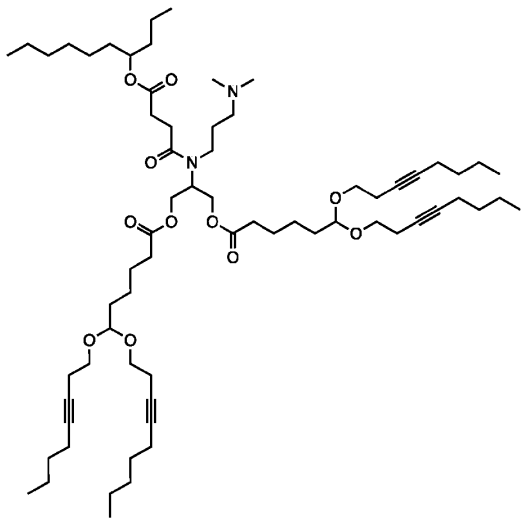
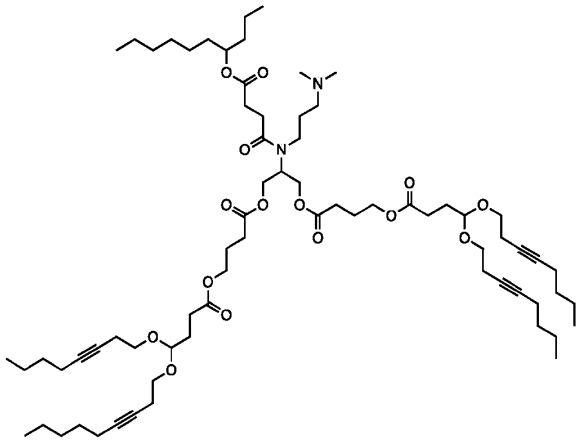
A-6		2-(4-(decan-4-yloxy)-4-oxo-N-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-7		2-(4-(decan-4-yloxy)-N-(3-morpholinopropyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)

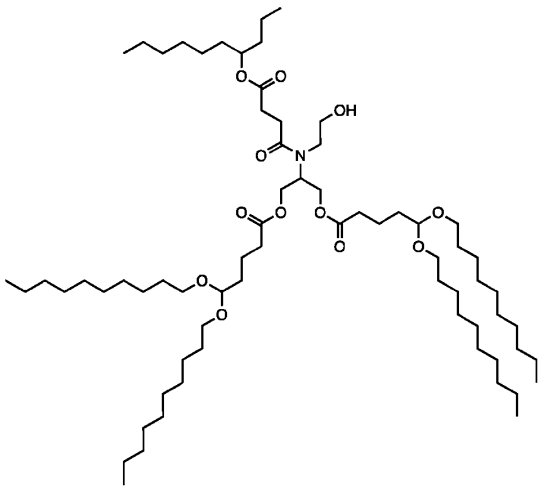
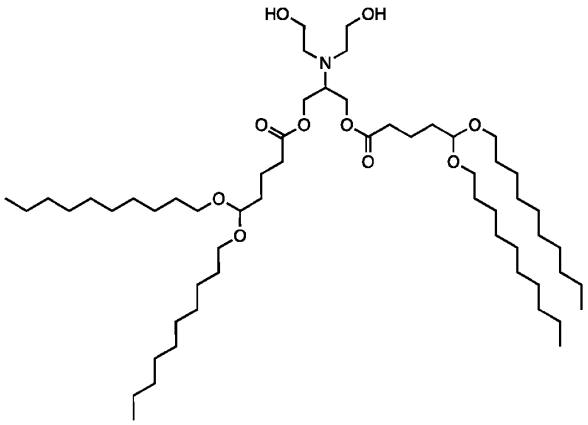
A-8		((2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(4,4-bis(octyloxy)butanoate)
A-9		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(5-((4,4-bis(octyloxy)butanoyl)oxy)pentanoate)

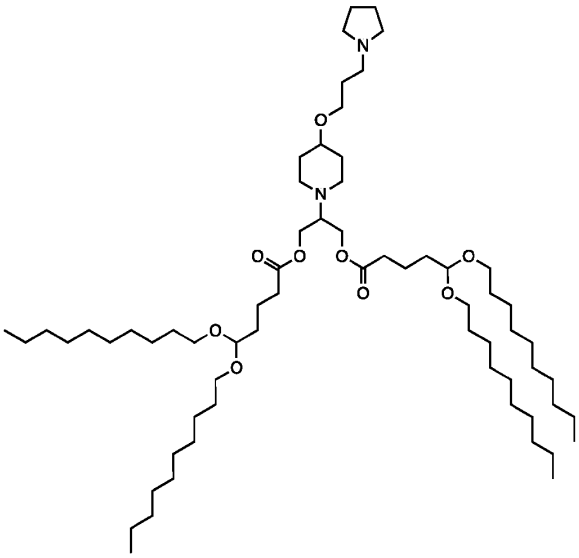
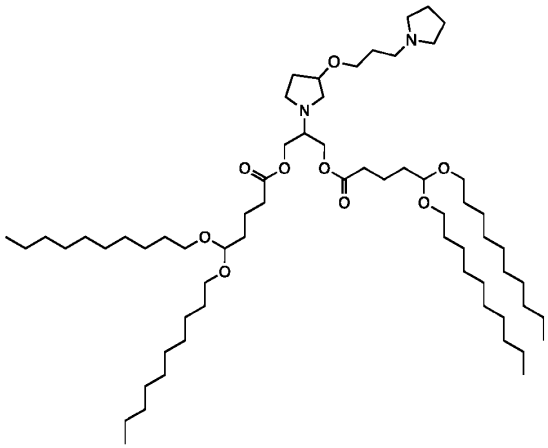
A-10		1-(3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl) 8-heptyl octanedioate
A-11		3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl (Z)-pentadec-7-enoate

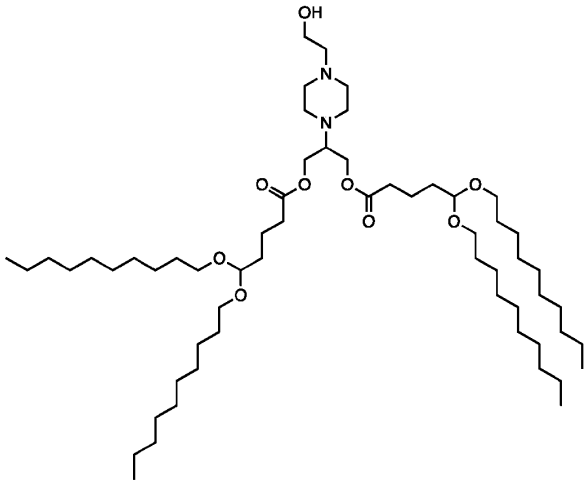
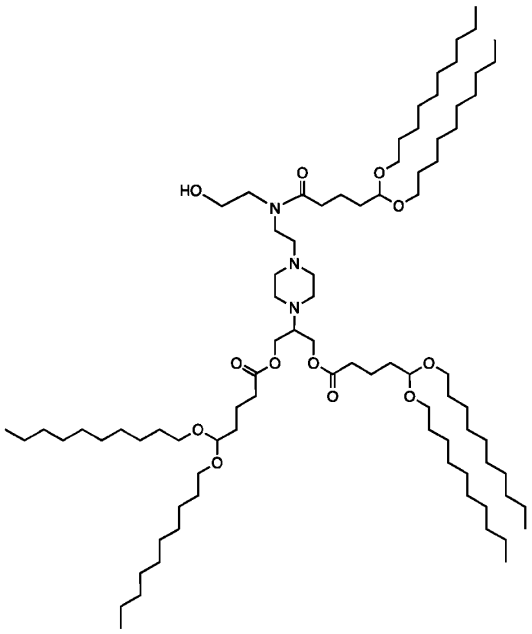
A-12		3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl (Z)-tridec-5-enoate
A-13		3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl oleate

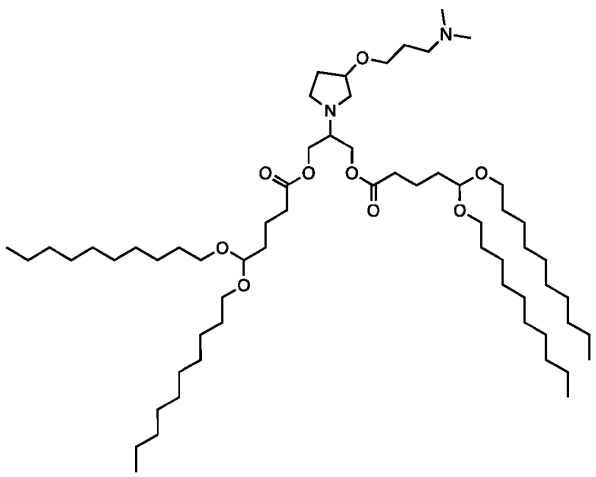
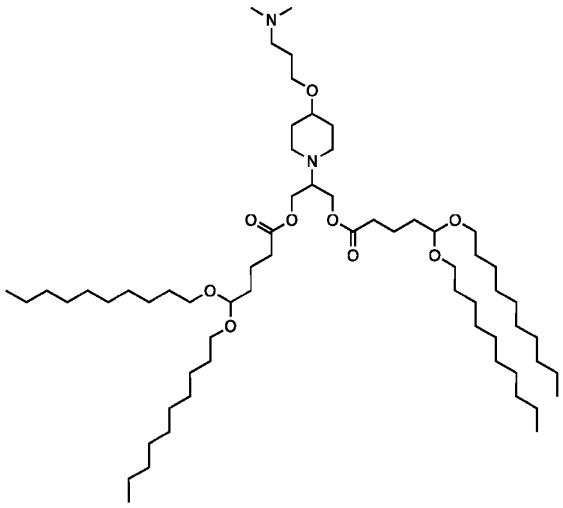
A-14		3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl (9Z,12Z)-octadeca-9,12-dienoate
A-15		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((Z)-dec-5-en-1-yl)oxy)hexanoate)

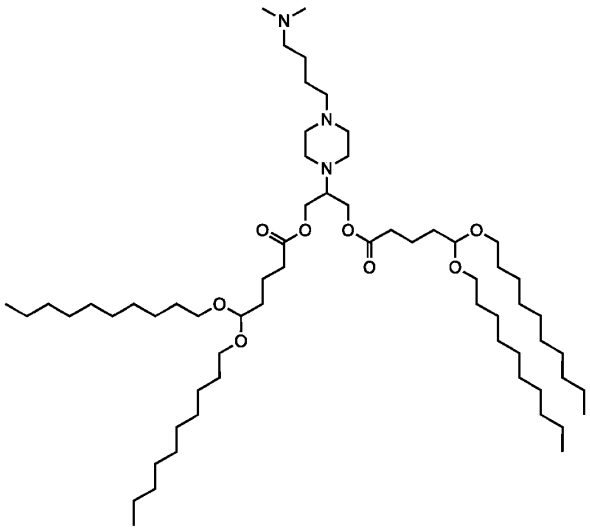
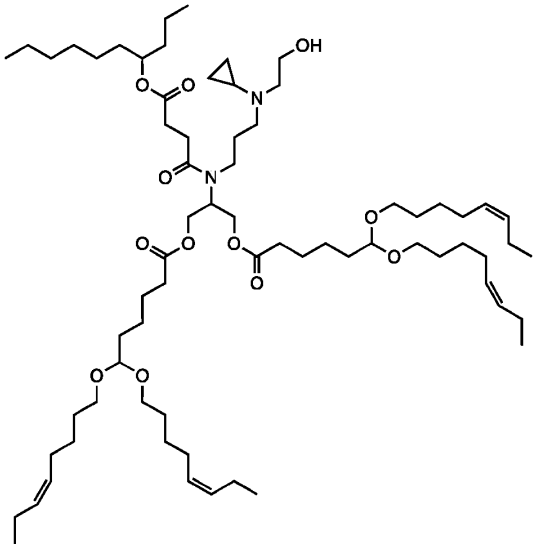
A-16		3-((6,6-bis(oct-3-yn-1-yloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl 6-(non-3-yn-1-yloxy)-6-(oct-3-yn-1-yloxy)hexanoate
A-17		decan-4-yl 4-((10,32-bis(oct-3-yn-1-yloxy)-13,18,24,29-tetraoxo-9,14,19,23,28,33-hexaoxadotetraconta-5,36-diyn-21-yl)(3-(dimethylamino)propyl)amino)-4-oxobutanoate

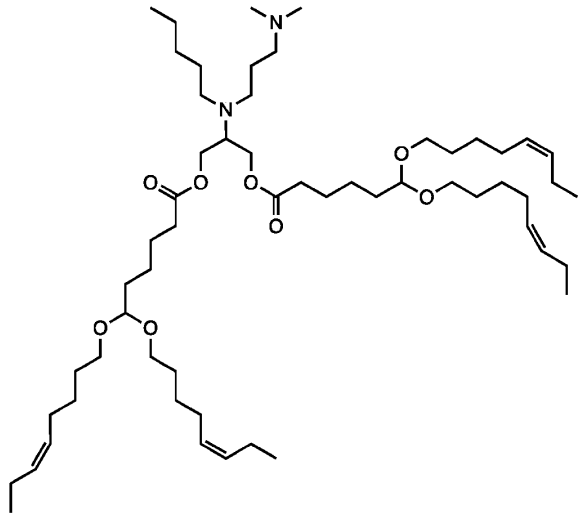
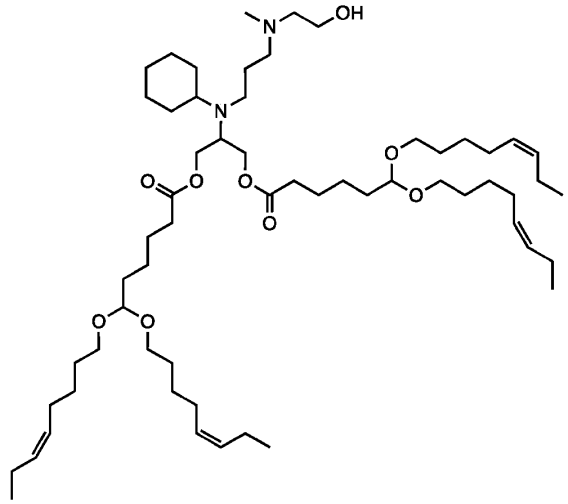
A-18		2-(4-(decan-4-yloxy)-N-(2-hydroxyethyl)-4-oxobutanamido)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-19		2-(bis(2-hydroxyethyl)amino)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

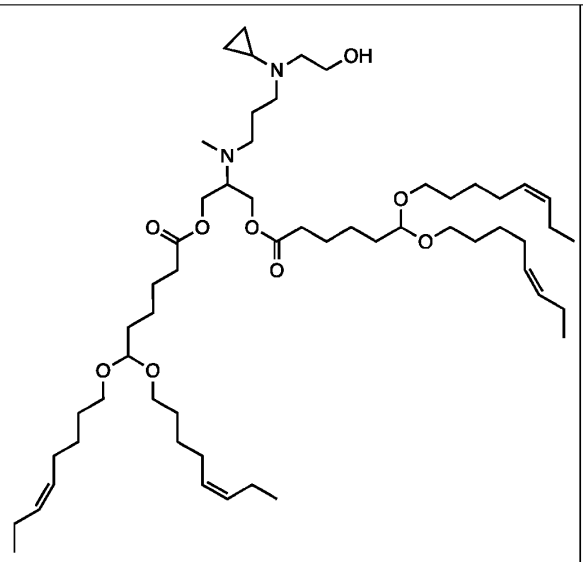
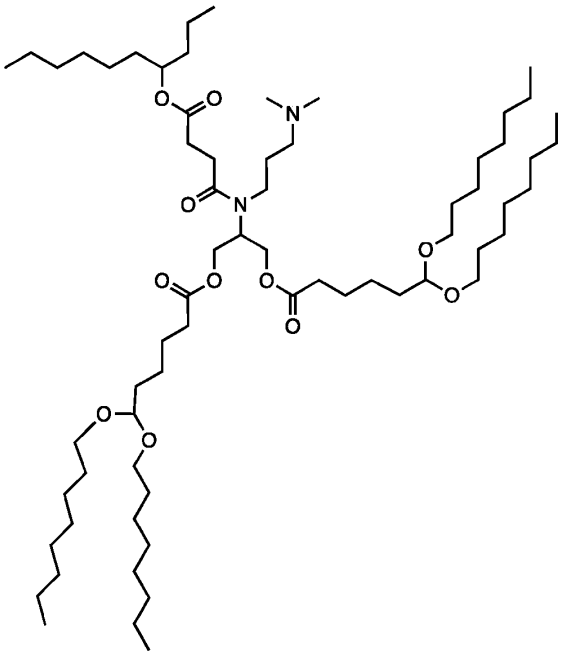
A-20		2-(4-(3-(pyrrolidin-1-yl)propoxy)piperidin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-21		2-(3-(3-(pyrrolidin-1-yl)propoxy)pyrrolidin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

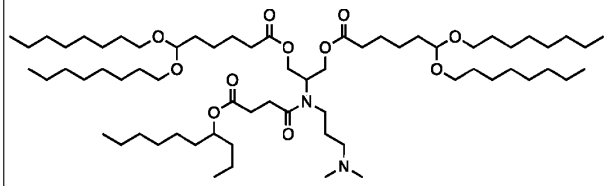
A-22		2-(4-(2-hydroxyethyl)piperazin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-23		2-(4-(2-(5,5-bis(decyloxy)-N-(2-hydroxyethyl)pentanamido)ethyl)piperazin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

A-32		2-(3-(3-(dimethylamino)propoxy)pyrrolidin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-24		2-(4-(3-(dimethylamino)propoxy)piperidin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

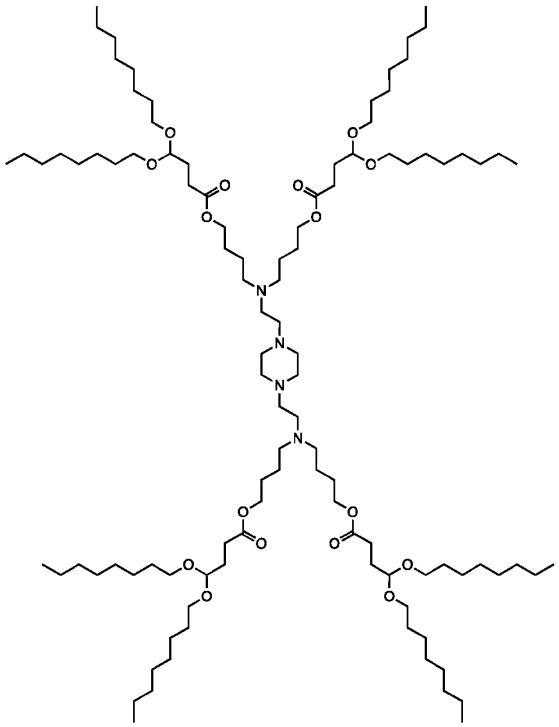
A-25		2-(4-(4-(dimethylamino)butyl)piperazin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-26		2-(N-(3-(cyclopropyl(2-hydroxyethyl)amino)propyl)-4-(decan-4-yloxy)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)

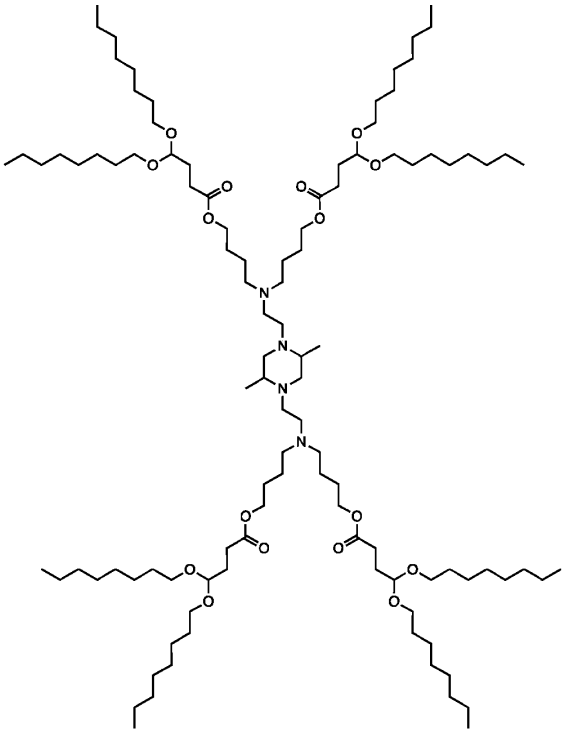
A-27		2-((3-(dimethylamino)propyl)(pentyl)amino)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)
A-28		2-(cyclohexyl(3-((2-hydroxyethyl)(methyl)amino)propyl)amino)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)

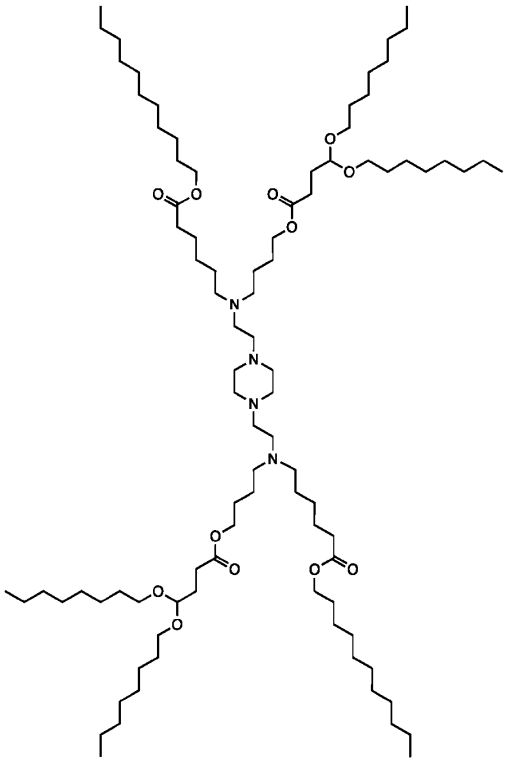
A-29		2-((3-(cyclopropyl(2-hydroxyethyl)amino)propyl)(methyl)amino)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)
A-30		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)

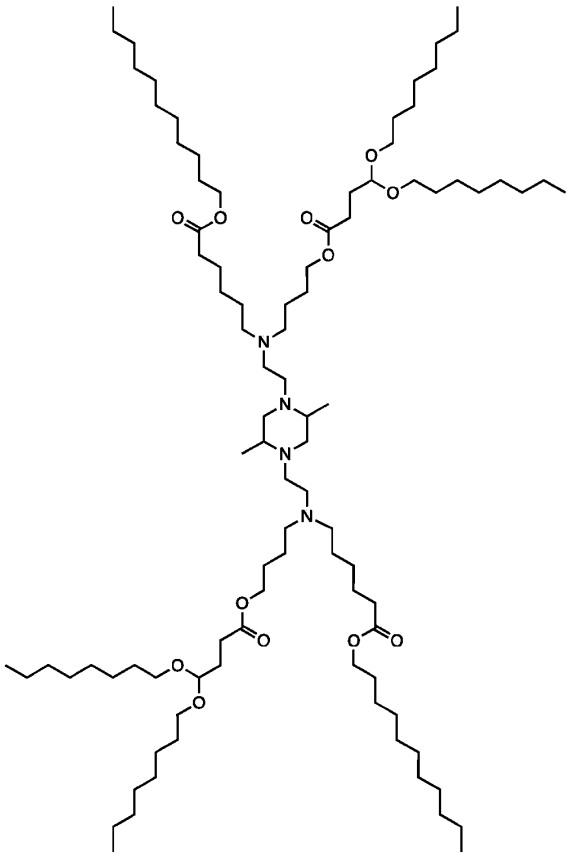
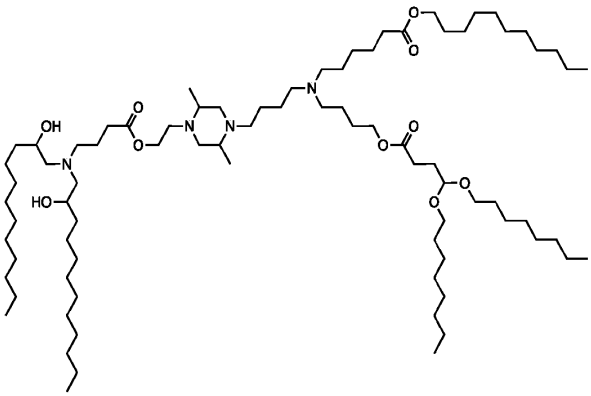
A-31		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)

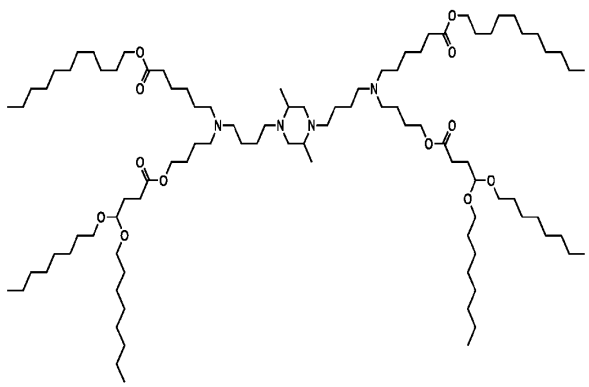
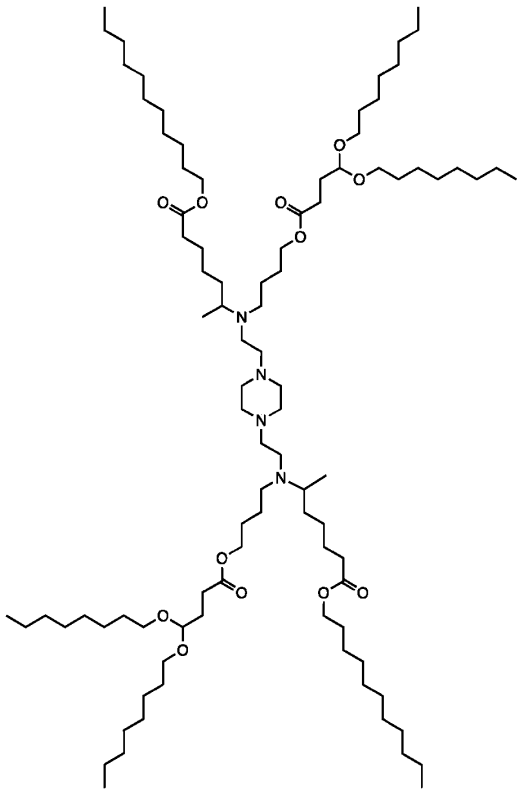
39. The compound of claim 8, having one of the following structures:

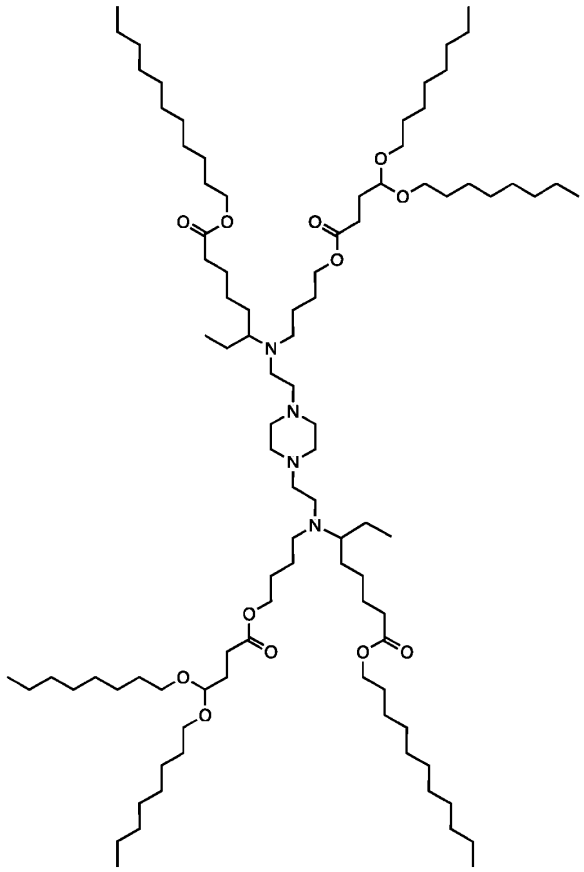
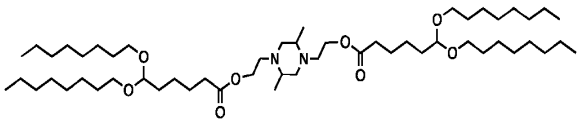
Compound	Structure	Name
B-1		((piperazine-1,4-diylbis(ethane-2,1-diyl))bis(azanetriyl))tetrakis(butane-4,1-diyl) tetrakis(4,4-bis(octyloxy)butanoate)

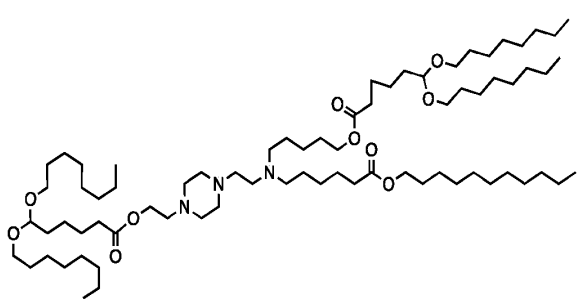
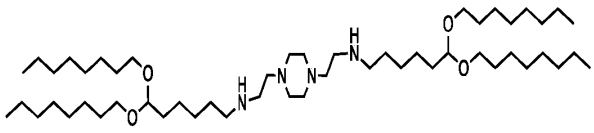
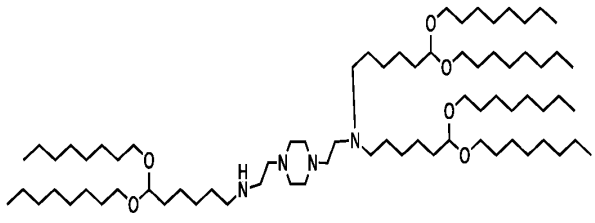
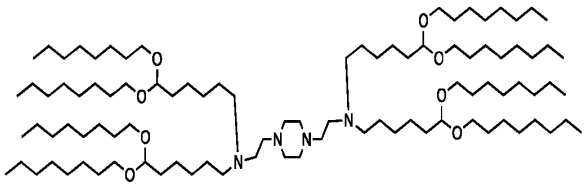
B-2		(((2,5-dimethylpiperazine-1,4-diyl)bis(ethane-2,1-diyl))bis(azanetriyl))tetrakis(butane-4,1-diyl) tetrakis(4,4-bis(octyloxy)butanoate)

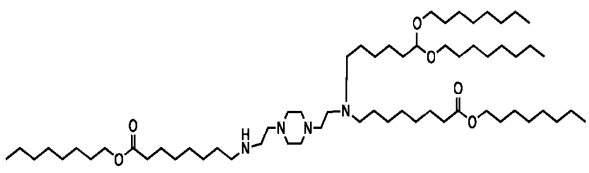
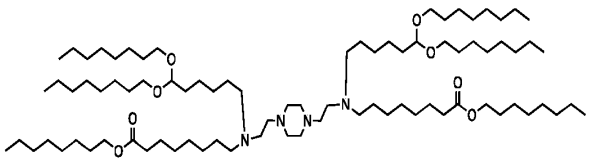
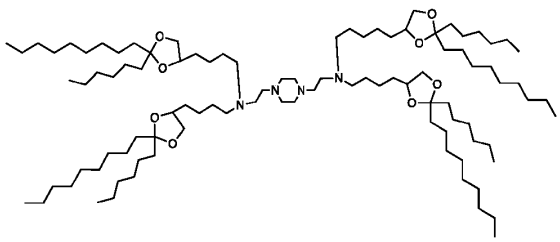
B-3		diundecyl 6,6'-((piperazine-1,4-diylbis(ethane-2,1-diyl))bis((4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)azanediyl))dihexanoate

B-4		diundecyl 6,6'-(((2,5-dimethylpiperazine-1,4-diyl)bis(ethane-2,1-diyl))bis((4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)azanediyl))dihexanoate
B-5		undecyl 6-((4-(4-(2-((4-(bis(2-hydroxydodecyl)amino)butanoyl)oxy)ethyl)-2,5-dimethylpiperazin-1-yl)butyl)(4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)amino)hexanoate

B-6		diundecyl 6,6'-(((2,5-dimethylpiperazine-1,4-diyl)bis(butane-4,1-diyl))bis((4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)azanediyl))dihexanoate
B-7		diundecyl 6,6'-((piperazine-1,4-diylbis(ethane-2,1-diyl))bis((4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)azanediyl))diheptanoate

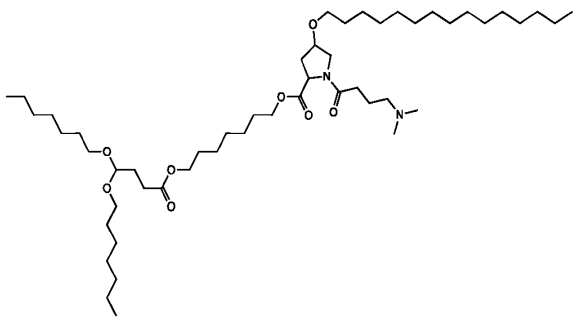
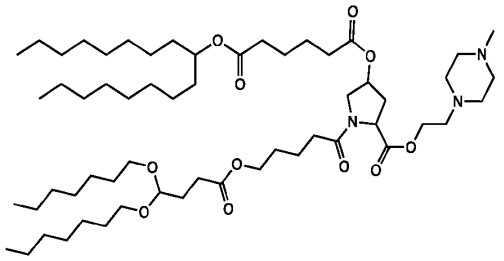
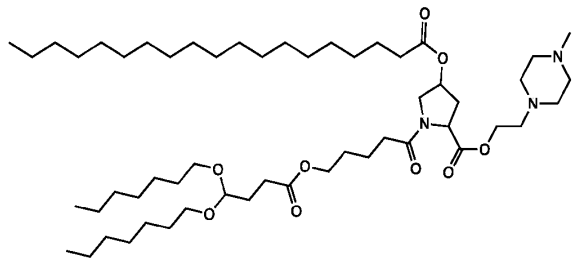
B-8		diundecyl 6,6'-((piperazine-1,4-diylbis(ethane-2,1-diyl))bis((4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)azanediyl))dioctanoate
B-9		(2,5-dimethylpiperazine-1,4-diyl)bis(ethane-2,1-diyl) bis(6,6-bis(octyloxy)hexanoate)

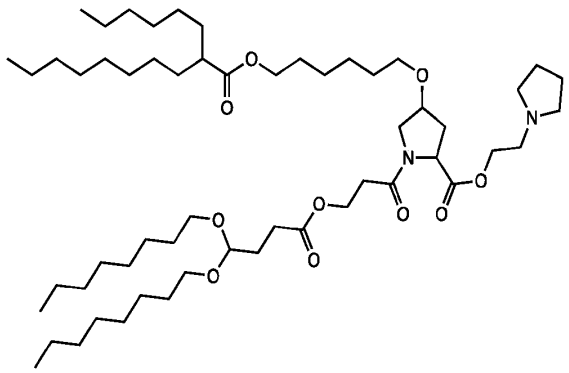
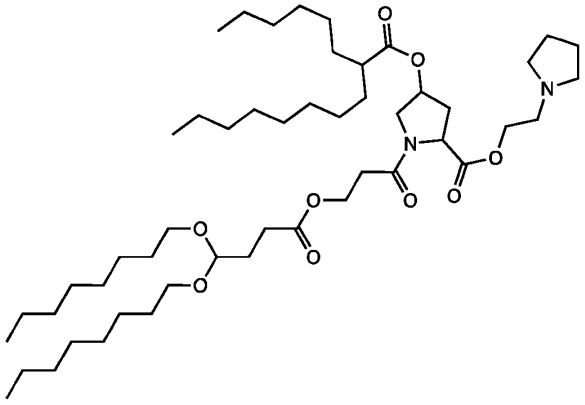
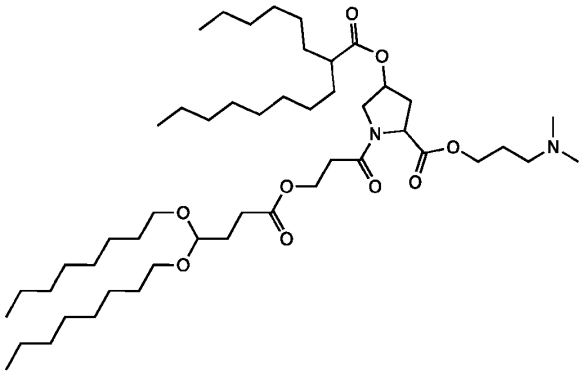
B-10		5-((2-(4-(2-((6,6-bis(octyloxy)hexanoyl)oxy)ethyl)piperazin-1-yl)ethyl)(6-oxo-6-(undecyloxy)hexyl)amino)pentyl 6,6-bis(octyloxy)hexanoate
B-11		N,N'-(piperazine-1,4-diylbis(ethane-2,1-diyl))bis(6,6-bis(octyloxy)hexan-1-amine)
B-12		N-(6,6-bis(octyloxy)hexyl)-N-(2-(4-(2-((6,6-bis(octyloxy)hexyl)amino)ethyl)piperazin-1-yl)ethyl)-6,6-bis(octyloxy)hexan-1-amine
B-13		N,N'-(piperazine-1,4-diylbis(ethane-2,1-diyl))bis(N-(6,6-bis(octyloxy)hexyl)-6,6-bis(octyloxy)hexan-1-amine)

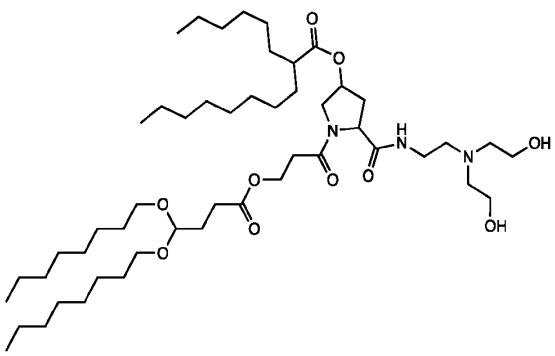
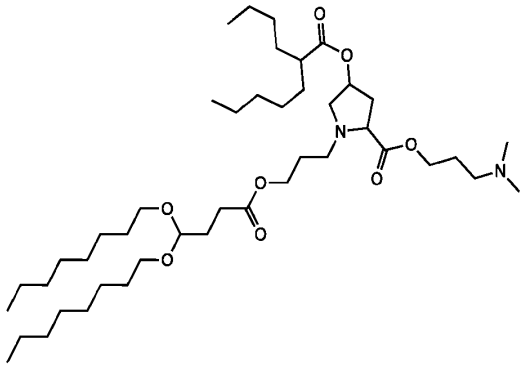
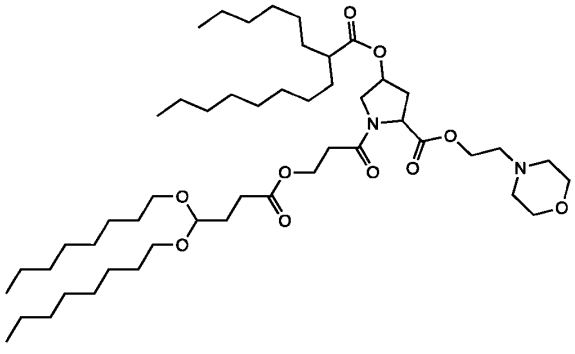
B-14		octyl 8-((6,6-bis(octyloxy)hexyl)(2-(4-(2-((8-(octyloxy)-8-oxooctyl)amino)ethyl)piperazin-1-yl)ethyl)amino)octanoate
B-15		dioctyl 8,8'-((piperazine-1,4-diylbis(ethane-2,1-diyl))bis((6,6-bis(octyloxy)hexyl)azanediyl))dioctanoate
B-16		N-(2-(4-(2-(bis(4-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)butyl)amino)ethyl)piperazin-1-yl)ethyl)-5-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)-N-(4-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)butyl)pentan-1-amine

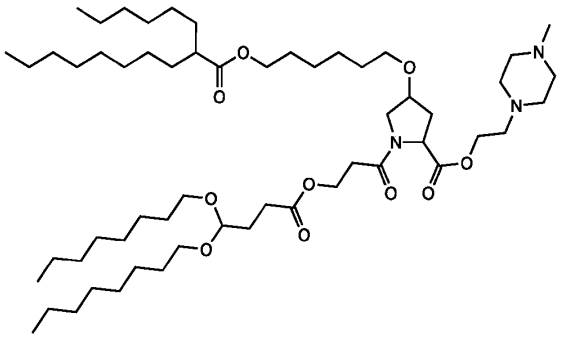
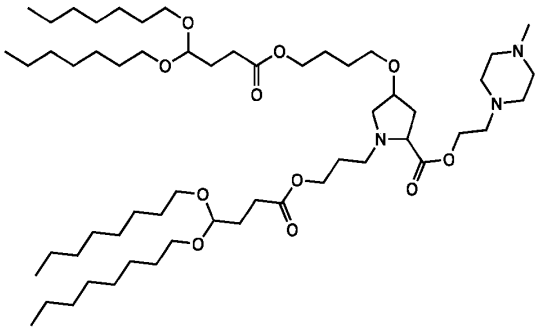
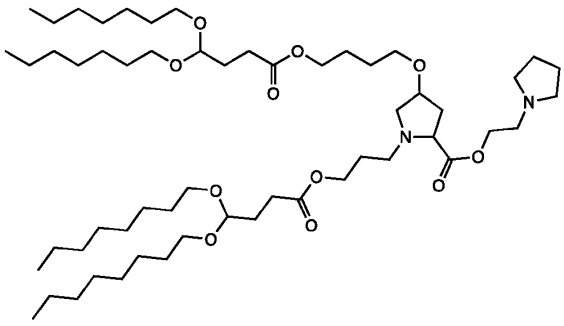
40. The compound of claim 15, having one of the following structures:

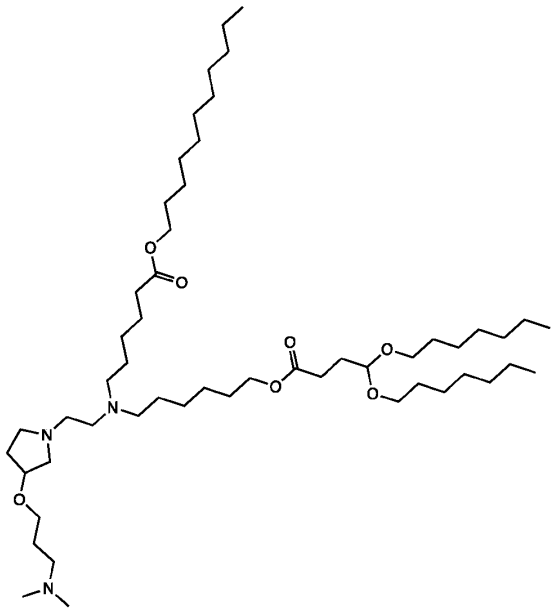
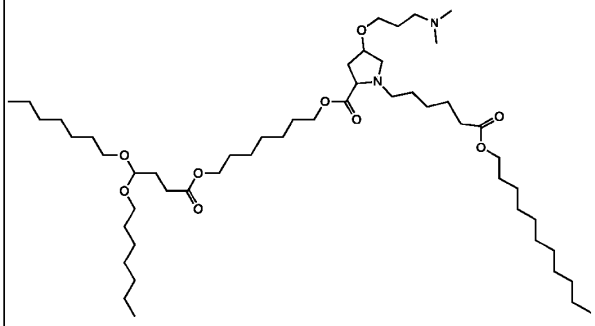
Compound	**Structure**	**Name**

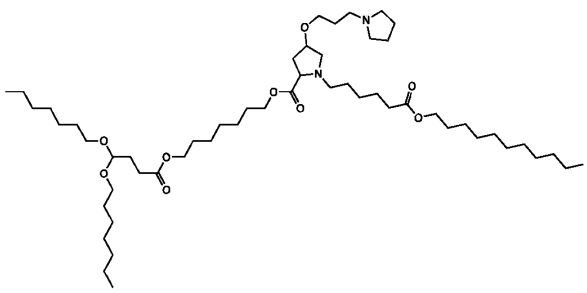
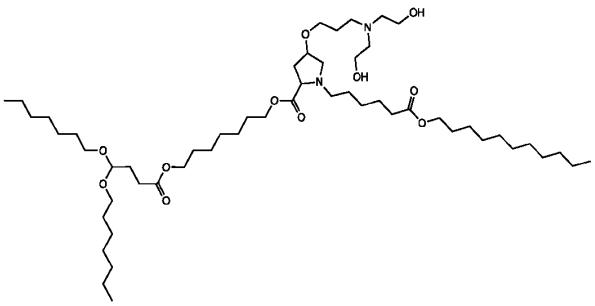
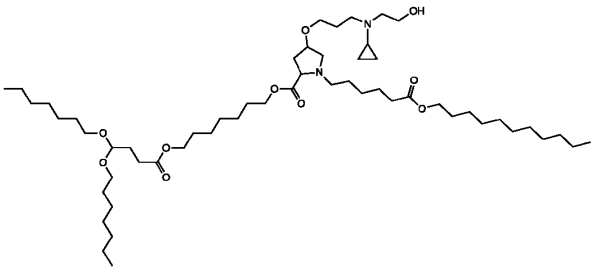
C-1		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 1-(4-(dimethylamino)butanoyl)-4-(pentadecyloxy)pyrrolidine-2-carboxylate
C-2		1-(5-((4,4-bis(heptyloxy)butanoyl)oxy)penta oyl)-5-((2-(4-methylpiperazin-1-yl)ethoxy)carbonyl)pyrrolidin-3-yl heptadecan-9-yl adipate
C-3		2-(4-methylpiperazin-1-yl)ethyl 1-(5-((4,4-bis(heptyloxy)butanoyl)oxy)penta oyl)-4-(nonadecanoyloxy)pyrrolidine-2-carboxylate

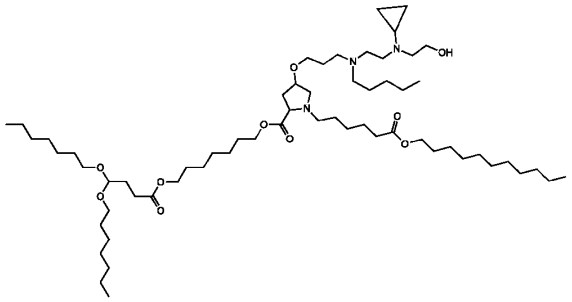
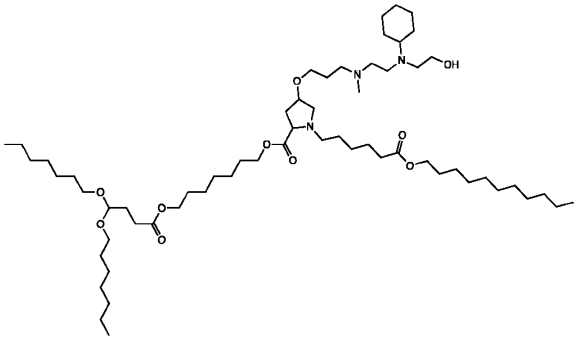
C-4		2-(pyrrolidin-1-yl)ethyl 1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propanyl)-4-((6-((2-hexyldecanoyl)oxy)hexyl)oxy)pyrrolidine-2-carboxylate
C-5		2-(pyrrolidin-1-yl)ethyl 1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propanyl)-4-((2-hexyldecanoyl)oxy)pyrrolidine-2-carboxylate
C-6		3-(dimethylamino)propyl 1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propanyl)-4-((2-hexyldecanoyl)oxy)pyrrolidine-2-carboxylate

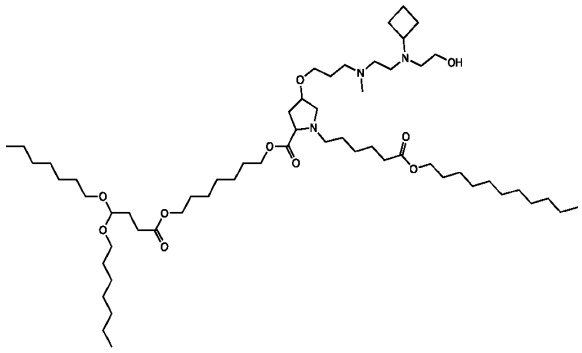
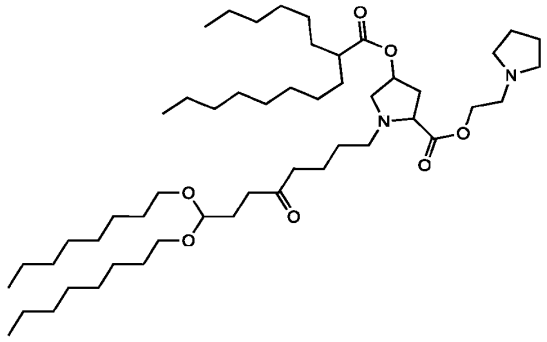
C-7		5-((2-(bis(2-hydroxyethyl)amino)ethyl)carbamyl)-1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propanyl)pyrrolidin-3-yl 2-hexyldecanoat
C-8		3-(dimethylamino)propyl 1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propyl)4-((2-butylheptanoyl)oxy)pyrrolidine-2-carboxylate
C-9		2-morpholinoethyl 1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propanyl)-4-((2-hexyldecanoyl)oxy)pyrrolidine-2-carboxylate

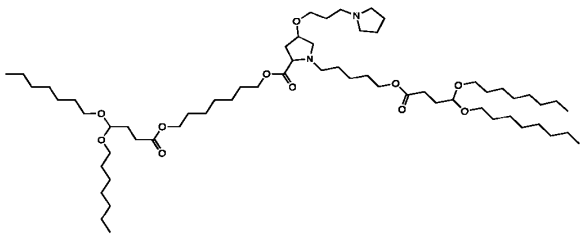
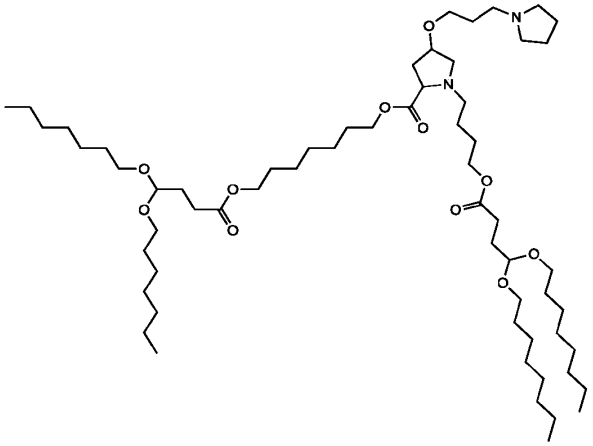
C-10		2-(4-methylpiperazin-1-yl)ethyl 1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propanyl)-4-((6-((2-hexyldecanoyl)oxy)hexyl)oxy)pyrolidine-2-carboxylate
C-11		2-(4-methylpiperazin-1-yl)ethyl 4-(4-((4,4-bis(heptyloxy)butanoyl)oxy)butox)-1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propyl)yrrolidine-2-carboxylate
C-12		2-(pyrrolidin-1-yl)ethyl 4-(4-((4,4-bis(heptyloxy)butanoyl)oxy)butox)-1-(3-((4,4-bis(octyloxy)butanoyl)oxy)propyl)yrrolidine-2-carboxylate

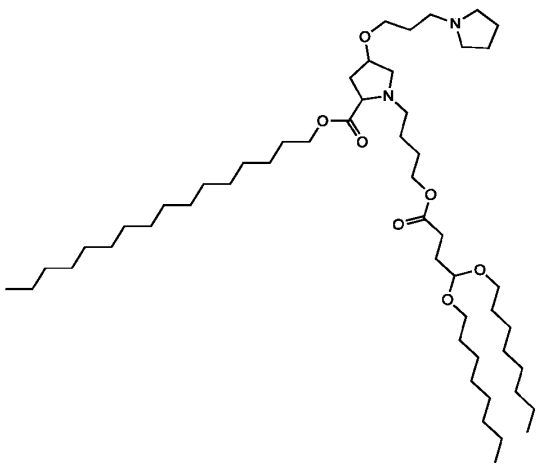
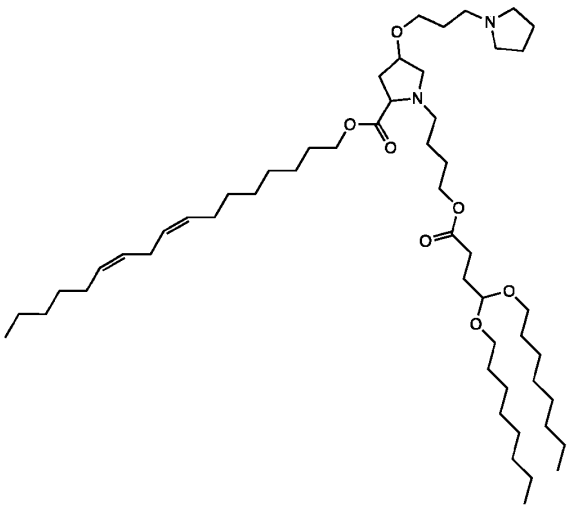
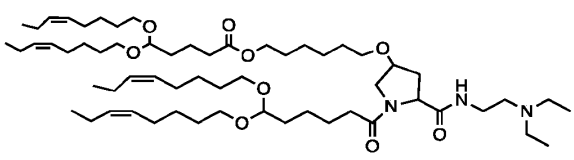
C-13		undecyl 6-((6-((4,4-bis(heptyloxy)butanoyl)oxy)hexyl 2-(3-(3-(dimethylamino)propoxy)pyrrolidi -1-yl)ethyl)amino)hexanoate
C-14		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 4-(3-(dimethylamino)propoxy)-1-(6-oxo-6-(undecyloxy)hexyl)pyrrolidine-2-carboxylate

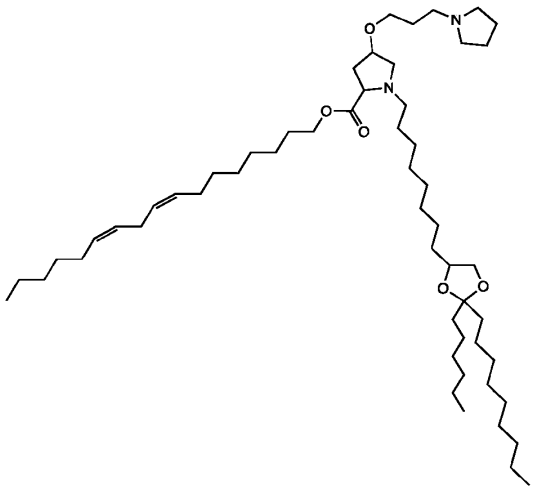
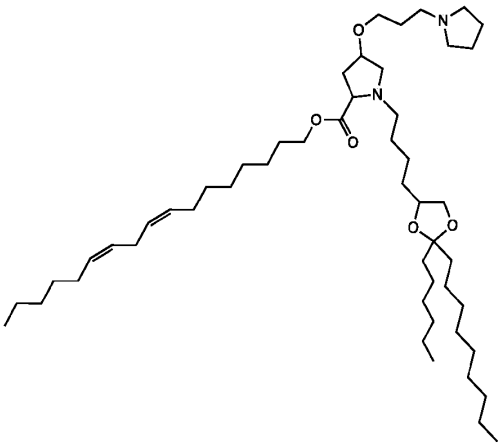
C-15		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 1-(6-oxo-6-(undecyloxy)hexyl)-4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate
C-16		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 4-(3-(bis(2-hydroxyethyl)amino)propoxy)-1-(oxo-6-(undecyloxy)hexyl)pyrrolidine-2-carboxylate
C-17		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 4-(3-(cyclopropyl(2-hydroxyethyl)amino)propoxy)-1-(oxo-6-(undecyloxy)hexyl)pyrrolidine-2-carboxylate

C-18		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 4-(3-((2-(cyclopropyl(2-hydroxyethyl)amino)ethyl)(pentyl mino)propoxy)-1-(6-oxo-6-(undecyloxy)hexyl)pyrrolidine-2-carboxylate
C-19		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 4-(3-((2-(cyclohexyl(2-hydroxyethyl)amino)ethyl)(methyl amino)propoxy)-1-(6-oxo-6-(undecyloxy)hexyl)pyrrolidine-2-carboxylate

C-20		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 4-(3-((2-(cyclobutyl(2-hydroxyethyl)amino)ethyl)(methyl amino)propoxy)-1-(6-oxo-6-(undecyloxy)hexyl)pyrrolidine-2-carboxylate
C-21		2-(pyrrolidin-1-yl)ethyl 1-(8,8-bis(octyloxy)-5-oxooctyl)-4-((2-hexyldecanoyl)oxy)pyrrolidine-2-carboxylate

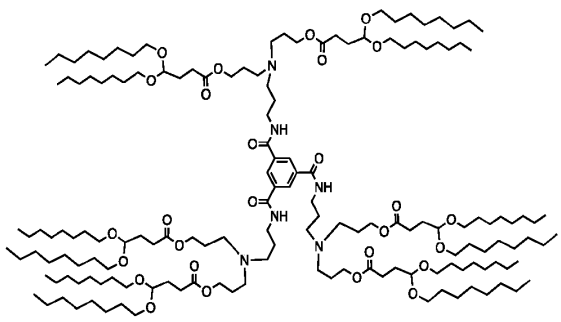
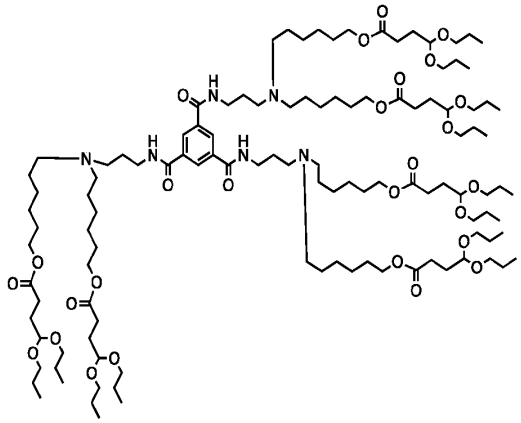
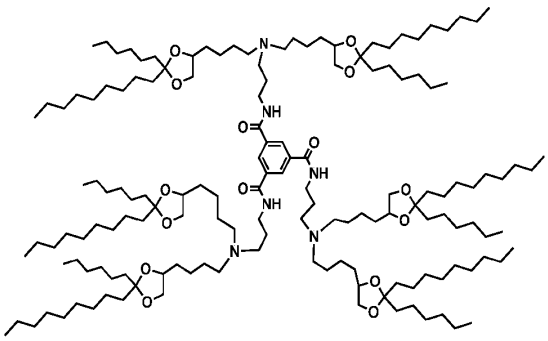
C-22		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 1-(5-((4,4-bis(octyloxy)butanoyl)oxy)pentyl) 4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate
C-23		7-((4,4-bis(heptyloxy)butanoyl)oxy)hepty 1-(4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)- 4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate

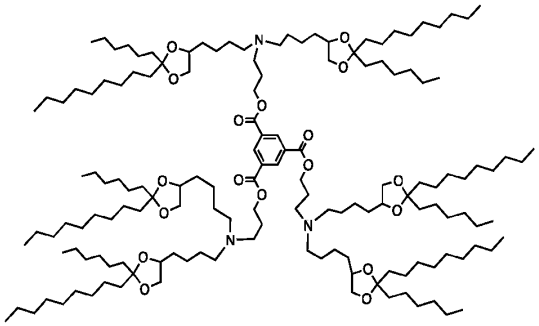
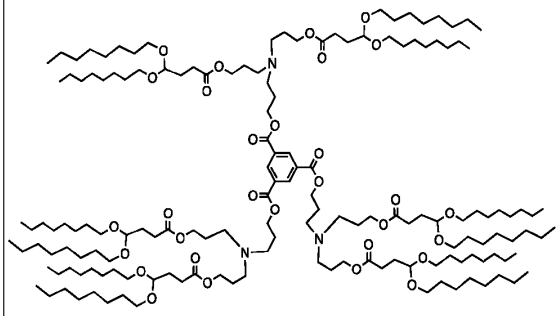
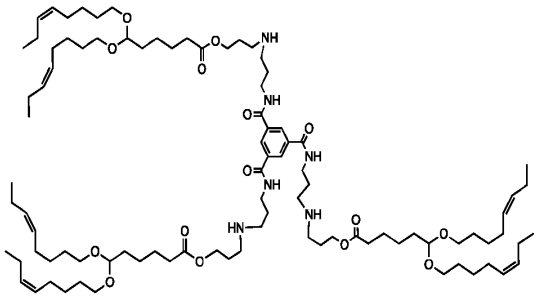
C-24		hexadecyl 1-(4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)-4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate
C-25		(8Z,11Z)-heptadeca-8,11-dien-1-yl 1-(4-((4,4-bis(octyloxy)butanoyl)oxy)butyl)-4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate
C-26		6-((1-(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoyl)-5-((2-(diethylamino)ethyl)carbamoyl)pyrrolidin-3-yl)oxy)hexyl 5,5-bis(((Z)-oct-5-en-1-yl)oxy)pentanoate

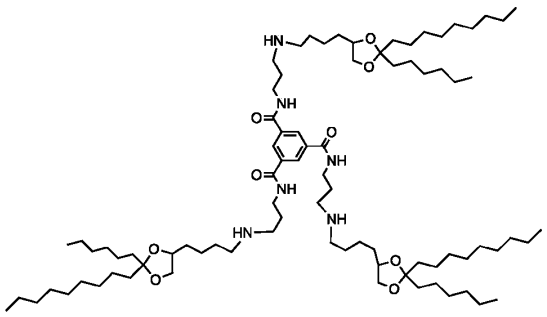
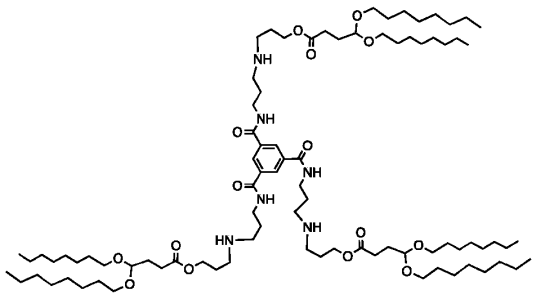
C-27		(8Z,11Z)-heptadeca-8,11-dien-1-yl 1-(8-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)octyl)-4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate
C-28		(8Z,11Z)-heptadeca-8,11-dien-1-yl 1-(4-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)butyl)-4-(3-(pyrrolidin-1-yl)propoxy)pyrrolidine-2-carboxylate

41. The compound of claim 28, having one of the following structures:

Compound	Structure	Name

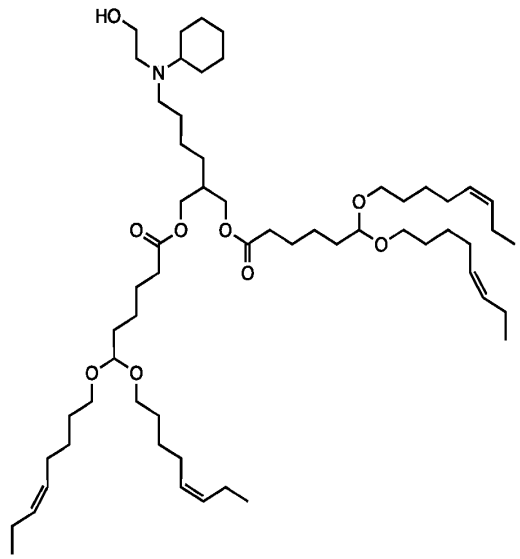
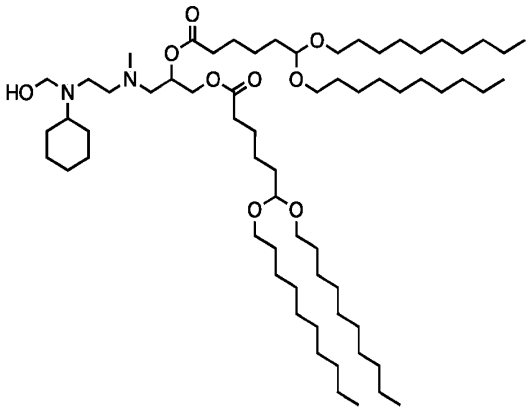
E-1		((((benzene-1,3,5-tricarbonyl)tris(azanediyl))tris(propane-3,1-diyl))tris(azanetriyl))hexakis(propane-3,1-diyl) hexakis(4,4-bis(octyloxy)butanoate)
E-2		((((benzene-1,3,5-tricarbonyl)tris(azanediyl))tris(propane-3,1-diyl))tris(azanetriyl))hexakis(hexane-6,1-diyl) hexakis(4,4-dipropoxybutanoate)
E-3		N1,N3,N5-tris(3-(bis(4-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)butyl)amino)propyl)benzene-1,3,5-tricarboxamide

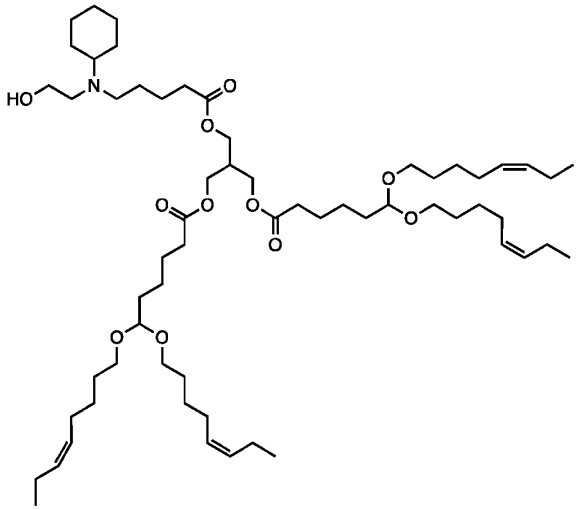
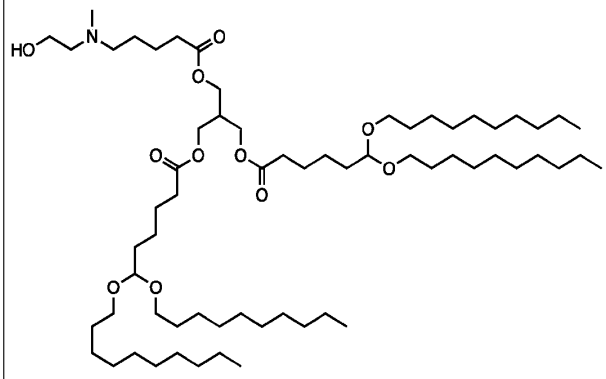
E-4		tris(3-(bis(4-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)butyl)amino)propyl) benzene-1,3,5-tricarboxylate
E-5		tris(3-(bis(3-((4,4-bis(octyloxy)butanoyl)oxy)propyl)amino)propyl) benzene-1,3,5-tricarboxylate
E-6		((((benzene-1,3,5-tricarbonyl)tris(azanediyl))tris(propane-3,1-diyl))tris(azanediyl))tris(propane-3,1-diyl) tris(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)

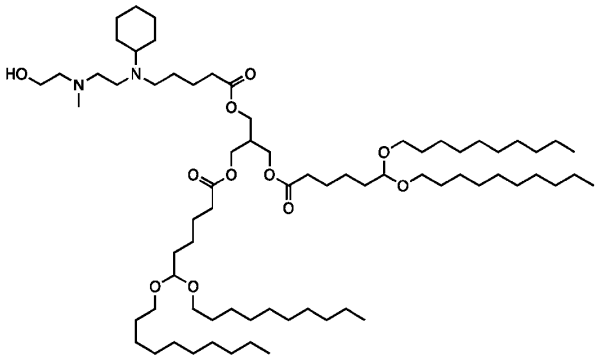
E-7		N1,N3,N5-tris(3-((4-(2-hexyl-2-nonyl-1,3-dioxolan-4-yl)butyl)amino)propyl)benzene-1,3,5-tricarboxamide
E-8		((((benzene-1,3,5-tricarbonyl)tris(azanediyl))tris(propane-3,1-diyl))tris(azanediyl))tris(propane-3,1-diyl) tris(4,4-bis(octyloxy)butanoate)

42. A compound having one of the following structures:

Compound	**Structure**	**Name**

D-1		2-(4-(cyclohexyl(2-hydroxyethyl)amino)butyl)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)
D-2		3-((2-(cyclohexyl(hydroxymethyl)amino)ethyl)(methyl)amino)propane-1,2-diyl bis(6,6-bis(decyloxy)hexanoate)

D-3		2-(((5-(cyclohexyl(2-hydroxyethyl)amino)pentanoyl)oxy)methyl)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)
D-4		2-(((5-((2-hydroxyethyl)(methyl)amino)pentanoyl)oxy)methyl)propane-1,3-diyl bis(6,6-bis(decyloxy)hexanoate)

D-5		2-(((5-(cyclohexyl(2-((2-hydroxyethyl)(methyl)amino)ethyl)amino)pentanoyl)oxy)methyl)propane-1,3-diyl bis(6,6-bis(decyloxy)hexanoate)

43. A lipid nanoparticle (LNP) comprising a lipid blend for targeted delivery of a nucleic acid into an immune cell, the lipid blend comprising:
(a) a lipid-immune cell targeting group conjugate comprising the compound of Formula (II): [Lipid] – [optional linker] – [immune cell targeting group]; and
(b) an ionizable cationic lipid comprising any one of the compound of claims 1 to 42, wherein the LNP further comprises a nucleic acid disposed therein.

44. The LNP of claim 43, wherein the immune cell targeting group comprises an antibody that binds a T cell antigen.

45. The LNP of claim 44, wherein the T cell antigen is CD3, CD4, CD7, CD8, or a combination thereof (e.g., both CD3 and CD8, both CD4 and CD8, or both CD7 and CD8).

46. The LNP of any one of claims 43 to 45, wherein the immune cell targeting group comprises an antibody that binds a Natural Killer (NK) cell antigen.

47. The LNP of claim 46, wherein the NK cell antigen is CD7, CD8, CD56, or a combination thereof (e.g., both CD7 and CD8).

48. The LNP of any one of claims 43 to 47, wherein the immune cell targeting group is covalently coupled to a lipid in the lipid blend via a polyethylene glycol (PEG) containing linker.

49. The LNP of claim 48, wherein the lipid covalently coupled to the immune cell targeting group via a PEG containing linker is distearoylglycerol (DSG), distearoyl-phosphatidylethanolamine (DSPE), dimyrstoyl-phosphatidylethanolamine (DMPE), distearoyl-glycero-phosphoglycerol (DSPG), dimyristoyl-glycerol (DMG), dipalmitoyl-phosphatidylethanolamine (DPPE), dipalmitoyl-glycerol (DPG), or ceramide.

50. The LNP of claim 48 or 49, wherein the PEG is PEG 2000 or PEG 3400.

51. The LNP of any one of claims 43 to 50, wherein the lipid-immune cell targeting group conjugate is present in the lipid blend in a range of 0.001 to 0.5 mole percent (e.g., 0.002-0.2 mole percent).

52. The LNP of any one of claims 43 to 51, wherein the lipid blend further comprises one or more of a structural lipid (e.g., a sterol), a neutral phospholipid, and a free PEG-lipid.

53. The LNP of any one of claims 43 to 52, wherein the ionizable cationic lipid is present in the lipid blend in a range of 30-70 (e.g., 40-60) mole percent.

54. The LNP of claim 52, wherein the sterol is present in the lipid blend in a range of 20-70 (e.g., 30-50) mole percent.

55. The LNP of claim 52 or 54, wherein the sterol is cholesterol.

56. The LNP of any one of claims 52 to 55, wherein the neutral phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and sphingomyelin.

57. The LNP of any one of claims 52 to 56, wherein the neutral phospholipid is present in the lipid blend in a range of 5-15 mole percent.

58. The LNP of any one of claims 52 to 57, wherein the free PEG-lipid is selected from the group consisting of PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, and PEG-modified dialkylglycerols, or wherein the PEG lipid is PEG-dioleoylgylcerol (PEG-DOG), PEG-dimyristoyl-glycerol (PEG-DMG), PEG-dipalmitoyl-glycerol (PEG-DPG), PEG-dilinoleoyl-glycero-phosphatidyl ethanolamine (PEG-DLPE), PEG-dimyrstoyl-phosphatidylethanolamine (PEG-DMPE), PEG-dipalmitoyl-phosphatidylethanolamine (PEG-DPPE), PEG-distearoylglycerol (PEG-DSG), PEG-diacylglycerol (PEG-DAG, e.g., PEG-DMG, PEG-DPG, and PEG-DSG), PEG-ceramide, PEG-distearoyl-glycero-phosphoglycerol (PEG-DSPG), PEG-dioleoyl-glycero-phosphoethanolamine (PEG-DOPE), 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, or a PEG-distearoyl-phosphatidylethanolamine (PEG-DSPE) lipid.

59. The LNP of any one of claims 52 to 57, wherein the free PEG-lipid comprises a diacylphosphatidylethanolamine comprising Dipalmitoyl (C16) chain or Distearoyl (C18) chain, and optionally the free PEG-lipid comprises PEG-DPG and PEG-DMG.

60. The LNP of any one of claims 52 to 59, wherein the free PEG-lipid is present in the lipid blend in a range of 1-4 mole percent.

61. The LNP of any one of claims 52 to 60, wherein the free PEG-lipid comprises the same or a different lipid as the lipid in the lipid-immune cell targeting group conjugate.

62. The LNP of any one of claims 43 to 61, wherein the LNP has a mean diameter in the range of 50-200 nm.

63. The LNP of claim 62, where the LNP has a mean diameter of about 100 nm.

64. The LNP of any one of claims 43 to 63, wherein the LNP has a polydispersity index in a range from 0.05 to 1.

65. The LNP of any one of claims 43 to 64, wherein the LNP has a zeta potential of from about +10 mV to about + 30 mV at pH 5.

66. The LNP of any one of claims 43 to 65, wherein the nucleic acid is DNA or RNA.

67. The LNP of claim 66, wherein the RNA is an mRNA.

68. The LNP of claim 67, wherein the mRNA encodes a receptor, a growth factor, a hormone, a cytokine, an antibody, an antigen, an enzyme, or a vaccine.

69. The LNP of claim 67, wherein the mRNA encodes a polypeptide capable of regulating immune response in the immune cell.

70. The LNP of claim 67, wherein the mRNA encodes a polypeptide capable of reprogramming the immune cell.

71. The LNP of claim 69, wherein the mRNA encodes a synthetic T cell receptor (synTCR) or a Chimeric Antigen Receptor (CAR).

72. The LNP of any one of claims 43 to 71, wherein the immune cell targeting group comprises an antibody, and the antibody is a Fab or an immunoglobulin single variable domain (e.g., a Nanobody).

73. The LNP of any one of claims 43 to 71, wherein the immune cell targeting group comprises a Fab, F(ab')2, Fab'-SH, Fv, or scFv fragment.

74. The LNP of claim 72 or claim 73, wherein the immune cell targeting group comprises a Fab that is engineered to knock out the natural interchain disulfide bond at the C-terminus.

75. The LNP of claim 74, wherein the Fab comprises a heavy chain fragment that comprises C233S substitution, and a light chain fragment that comprises C214S substitution, numbering according to Kabat.

76. The LNP of any one of claims 73 to 75, wherein the immune cell targeting group comprises a Fab that has a non-natural interchain disulfide bond (e.g., an engineered, buried interchain disulfide bond).

77. The LNP of claim 76, wherein the Fab comprises F174C substitution in the heavy chain fragment, and S176C substitution in the light chain fragment, numbering according to Kabat.

78. The LNP of claims 73 to 77, wherein the immune cell targeting group comprises a Fab that comprises a cysteine at the C-terminus of the heavy or light chain fragment.

79. The LNP of claim 78, wherein the Fab further comprises one or more amino acids between the heavy chain fragment of the Fab and the C-terminal cysteine.

80. The LNP of claim 72, wherein the immune cell targeting group comprises an immunoglobulin single variable domain.

81. The LNP of claim 72 or 80, wherein the immunoglobulin single variable domain comprises a cysteine at the C-terminus.

82. The LNP of claim 81, wherein the immunoglobulin single variable domain comprises a V_{HH} domain and further comprises a spacer comprising one or more amino acids between the V_{HH} domain and the C-terminal cysteine.

83. The LNP of any one of claims 73 and 80 to 82, wherein the immune cell targeting group comprises two or more V_{HH} domains.

84. The LNP of claim 83, wherein the two or more V_{HH} domains are linked by an amino acid linker.

85. The LNP of claim 83, wherein the immune cell targeting group comprises a first V_{HH} domain linked to an antibody CH1 domain and a second V_{HH} domain linked to an antibody light chain constant domain, and wherein the antibody CH1 domain and the antibody light chain constant domain are linked by one or more disulfide bonds.

86. The LNP of any one of claims 72 and 80 to 82, wherein the immune cell targeting group comprises a V_{HH} domain linked to an antibody CH1 domain, and wherein the antibody CH1 domain is linked to an antibody light chain constant domain by one or more disulfide bonds.

87. The LNP of claim 85 or 86, wherein the CH1 domain comprises F174C and C233S substitutions, and the light chain constant domain comprises S176C and C214S substitutions, numbering according to Kabat.

88. The LNP of any one of claims 43 to 69, wherein the immune cell targeting group comprises a Fab that comprises:
(a) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 1 and a light chain fragment comprising the amino acid sequence of SEQ ID NO:2 or 3; or
(b) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 6 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 7.

89. The LNP of any one of claims 43 to 88, wherein the ionizable cationic lipid is selected from lipid A-1 to A-10.

90. The LNP of any one of claims 43 to 88, wherein the ionizable cationic lipid is selected from lipid A-11 to A-20.

91. The LNP of any one of claims 43 to 88, wherein the ionizable cationic lipid is selected from lipid A-21 to A-31.

92. The LNP of any one of claims 43 to 91, wherein the LNP comprises:
(a) the ionizable cationic lipid;
(b) the conjugate comprising the compound of the following formula:
[Lipid] – [optional linker] – [immune cell targeting group];
(c) a sterol or other structural lipid;
(d) a neutral phospholipid;
(e) a free Polyethylene glycol (PEG) lipid; and
(f) the nucleic acid.

93. The LNP of any one of claims 43 to 92, wherein the LNP is for delivering a nucleic acid into an immune cell, and wherein the immune cell is an NK cell, and the immune cell targeting group comprises an antibody that binds CD56.

94. The LNP of any one of claims 43 to 92, wherein the LNP is for delivering a nucleic acid into an immune cell, and wherein the immune cell targeting group comprises an antibody that binds CD7 or CD8, and the free PEG lipid is DMG-PEG or PEG-DPG.

95. The LNP of any one of claims 43 to 92, wherein the immune cell targeting group comprises an antibody, and the antibody is a Fab or an immunoglobulin single variable domain.

96. The LNP of claim 95, wherein the Fab is engineered to knock out the natural interchain disulfide at the C-terminus.

97. The LNP of claim 96, wherein the Fab comprises a heavy chain fragment that comprises C233S substitutions, and a light chain fragment that comprises C214S substitutions.

98. The LNP of claim 96, wherein the Fab comprises a non-natural interchain disulfide.

99. The LNP of claim 96, wherein the Fab comprises F174C substitution in the heavy chain fragment, and S176C substitution in the light chain fragment.

100. The LNP of claim 95, wherein the antibody is an immunoglobulin single variable (ISV) domain, and the ISV domain is an Nanobody® ISV.

101. The LNP of claim 100, wherein the free PEG lipid comprises a PEG having a molecular weight of at least 2000 daltons.

102. The LNP of claim 101, wherein the PEG has a molecular weight of about 3000 to 5000 daltons.

103. The LNP of claim 95, wherein the antibody is a Fab.

104. The LNP of claim 103, wherein the Fab binds CD3, and the free PEG lipid in the LNP comprises a PEG having a molecular weight of about 2000 daltons.

105. The LNP of claim 103, wherein the Fab is an anti-CD4 antibody, and the free PEG lipid in the LNP comprises a PEG having a molecular weight of about 3000 to 3500 daltons.

106. The LNP of claim 95, wherein the immune cell targeting group comprises two or more V_{HH} domains.

107. The LNP of claim 106, wherein the two or more V_{HH} domains are linked by an amino acid linker.

108. The LNP of claim 107, wherein the immune cell targeting group comprises a first V_{HH} domain linked to an antibody CH1 domain and a second V_{HH} domain linked to an antibody light chain constant domain.

109. The LNP of any one of claims 43 to 92, wherein the LNP is for delivering a nucleic acid into an immune cell, and wherein the LNP binds CD3, and also binds CD11a or CD18.

110. The LNP of claim 109, wherein the LNP comprises two conjugates, wherein the first conjugate comprises an antibody that binds CD3, and the second conjugate comprises an antibody that binds CD11a or CD18.

111. The LNP of claim 109, wherein the LNP comprises one conjugate, and the conjugate comprises a bispecific antibody that binds both CD3 and CD11a.

112. The LNP of claim 109, wherein the LNP comprises one conjugate, and the conjugate comprises a bispecific antibody that binds both CD3 and CD18.

113. The LNP of claim 111 or 112, wherein the bispecific antibody is an immunoglobulin single variable domain or Fab-ScFv.

114. The LNP of any one of claims 43 to 92, wherein the LNP is for delivering a nucleic acid into an immune cell, and wherein the LNP binds CD7 and CD8 of the immune cell.

115. The LNP of claim 114, wherein the LNP comprises two conjugates, wherein the first conjugate comprises an antibody that binds CD7, and a second conjugate that binds CD8.

116. The LNP of claim 114, wherein the LNP comprises one conjugate, wherein the conjugate comprises a bispecific antibody that binds CD7 and CD8.

117. The LNP of claim 116, wherein the bispecific antibody is an immunoglobulin single variable domain or Fab-ScFv.

118. The LNP of any one of claims 43 to 92, wherein the LNP binds to a first antigen on the surface of the immune cell which is a first type of immune cell, and also binds to a second antigen on the surface of a second type of immune cell.

119. The LNP of claim 118, wherein the two different types of immune cells are CD4+ T cells and CD8+ T cell.

120. The LNP of claim 118, wherein the LNP comprises two conjugates, and the first conjugate comprises a first antibody that binds to the first antigen of the first type of immune cell, and the second conjugate comprises a second antibody that binds to the second antigen of the second type of immune cell.

121. The LNP of claim 118, wherein the LNP comprises one conjugate, and the conjugate comprises a bispecific antibody, and the bispecific antibody binds to both the first antigen on the first type of immune cell, and the second antigen on the second type of immune cell.

122. The LNP of any one of claims 43 to 92, wherein the bispecific antibody is an immunoglobulin single variable domain or a Fab-ScFv.

123. The LNP of any one of claims 43 to 92, wherein the LNP is for delivering a nucleic acid into an immune cell, and wherein the immune cell targeting group comprises a single antibody that binds to CD3 or CD7.

124. The LNP of any one of claims 43 to 92, wherein the LNP is for delivering a nucleic acid into an immune cell, and wherein the immune cell targeting group binds to CD7, CD8, or both CD7 and CD8.

125. The LNP of any one of claims 43 to 92, wherein the LNP is for delivering a nucleic acid into both T cells and NK cells, wherein the immune cell targeting group binds to:
(a) both CD3 and CD56;
(b) both CD8 and CD56; or
(c) both CD7 and CD56.

126. The LNP of any one of claims 93 to 125, wherein the LNP has a mean diameter in the range of 50-200 nm.

127. The LNP of claim 126, where the LNP has a mean diameter of about 100 nm.

128. The LNP of any one of claims 93 to 127, wherein the LNP has a polydispersity index in a range from 0.05 to 1.

129. The LNP of any one of claims 93 to 128, wherein the LNP has a zeta potential of from about+10 mV to about + 30 mV at pH 5.

130. The LNP of any one of claims 93 to 129, wherein the nucleic acid is DNA or RNA.

131. The LNP of claim 130, wherein the RNA is an mRNA.

132. The LNP of claim 131, wherein the mRNA encodes a receptor, a growth factor, a hormone, a cytokine, an antibody, an antigen, an enzyme, or a vaccine.

133. The LNP of claim 131, wherein the mRNA encodes a polypeptide capable of regulating immune response in the immune cell.

134. The LNP of claim 133, wherein the mRNA encodes a polypeptide capable of reprogramming the immune cell.

135. The LNP of claim 134, wherein the mRNA encodes a synthetic T cell receptor (synTCR) or a Chimeric Antigen Receptor (CAR).

136. The LNP of any one of claims 43 to 92, wherein the LNP is for delivering a nucleic acid into an immune cell, and wherein the immune cell targeting group comprises a Fab lacking the native interchain disulfide bond.

137. The LNP of claim 136, wherein the Fab is engineered to replace one or both cysteines on the native constant light chain and the native constant heavy chain that form the native interchain disulfide with a non-cysteine amino acid, therefor to remove the native interchain disulfide bond in the Fab.

138. A method of targeting the delivery of a nucleic acid to an immune cell of a subject, comprising contacting the immune cell with the LNP of any one of claims 43 to 137, wherein the LNP comprises the nucleic acid.

139. A method of expressing a polypeptide of interest in a targeted immune cell of a subject, comprising contacting the immune cell with the LNP of any one of claims 43 to 137, wherein the LNP comprises a nucleic acid encoding the polypeptide.

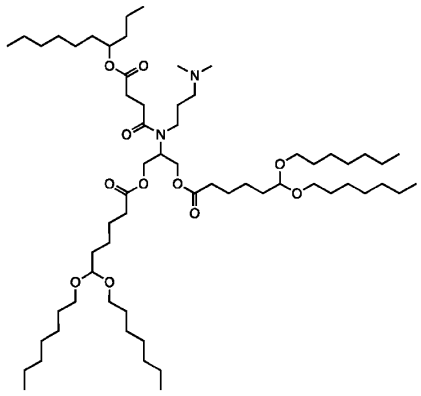
140. A method of modulating cellular function of a target immune cell of a subject, comprising administering to the subject the LNP of any one of claims 43 to 137, wherein the LNP comprises a nucleic acid modulates the cellular function of the immune cell.

141. A method of treating, ameliorating, or preventing a symptom of a disorder or disease in a subject in need thereof, comprising administering to the subject an LNP for delivering a nucleic acid into an immune cell of the subject, wherein the LNP is any one of claims 43 to 137, wherein the LNP comprises the nucleic acid.

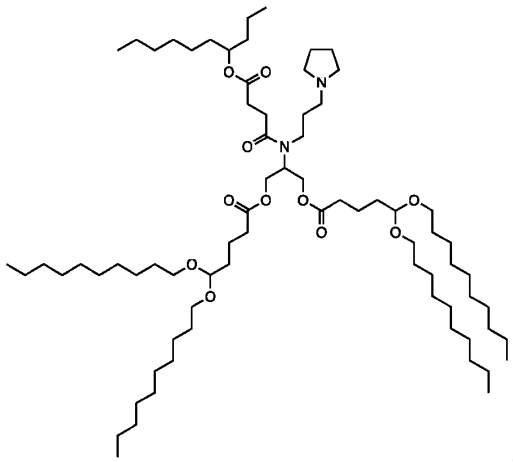
142. The method of claim 141, wherein the disorder is an immune disorder, an inflammatory disorder, or cancer.

143. The method of claim 141, wherein the nucleic acid encodes an antigen for use in a therapeutic or prophylactic vaccine for treating or preventing an infection by a pathogen.

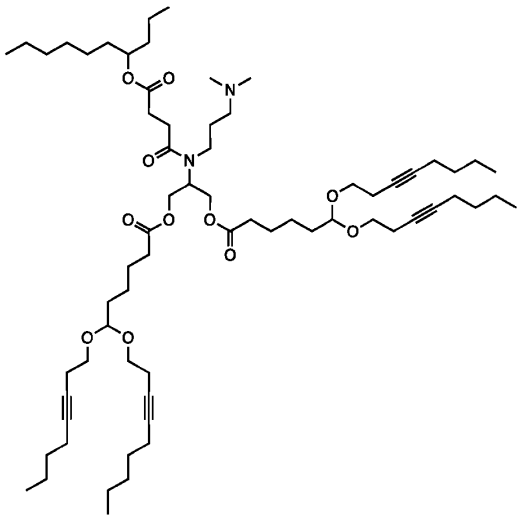
144. The method of any one of claims 138 to 143, wherein the ionizable cationic lipid is



145. The method of any one of claims 138 to 143, wherein the ionizable cationic lipid is



146. The method of any one of claims 138 to 143, wherein the ionizable cationic lipid is



147. The method of any one of claims 138 to 146, wherein the immune cell targeting group comprises an antibody that binds a T cell antigen.

148. The method of claim 147, wherein the T cell antigen is CD3, CD8, or both CD3 and CD8.

149. The method of any one of claims 138 to 146, wherein the immune cell targeting group comprises an antibody that binds a Natural Killer (NK) cell antigen.

150. The method of claim 149, wherein the NK cell antigen is CD7, CD8, or CD56.

151. The method of any one of claims 138 to 150, wherein the antibody is a human or humanized antibody.

152. The method of any one of claims 138 to 151, wherein the immune cell targeting group is covalently coupled to a lipid in the lipid blend via a polyethylene glycol (PEG) containing linker.

153. The method of claim 152, wherein the lipid covalently coupled to the immune cell targeting group via a PEG containing linker is distearoylglycerol (DSG), distearoyl-phosphatidylethanolamine (DSPE), dimyrstoyl-phosphatidylethanolamine (DMPE), distearoyl-

glycero-phosphoglycerol (DSPG), dimyristoyl-glycerol (DMG), dipalmitoyl-phosphatidylethanolamine (DPPE), dipalmitoyl-glycerol (DPG), or ceramide.

154. The method of claim 152 or 153, wherein the PEG is PEG 2000.

155. The method of any one of claims 138 to 154, wherein the lipid-immune cell targeting group conjugate is present in the lipid blend in a range of 0.002-0.2 mole percent.

156. The method of any one of claims 138 to 155, wherein the ionizable cationic lipid is present in the lipid blend in a range of 40-60 mole percent.

157. The method of claim 138 to 156, wherein the sterol is cholesterol.

158. The method of any one of claims 49 to 157, wherein the sterol is present in the lipid blend in a range of 30-50 mole percent.

159. The method of clam 138 to 158, wherein the neutral phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), sphingomyelin (SM).

160. The method of claim 138 to 159, wherein the neutral phospholipid is present in the lipid blend in a range of 5-15 mole percent.

161. The method of any one of claims 138 to 160, wherein the free PEG-lipid is selected from the group consisting of PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, and PEG-modified dialkylglycerols. For example, a PEG lipid may be PEG-dioleoylgylcerol (PEG-DOG), PEG-dimyristoyl-glycerol (PEG-DMG), PEG-dipalmitoyl-glycerol (PEG-DPG), PEG-dilinoleoyl-glycero-phosphatidyl ethanolamine (PEG-DLPE), PEG-dimyrstoyl-

phosphatidylethanolamine (PEG-DMPE), PEG-dipalmitoyl-phosphatidylethanolamine (PEG-DPPE), PEG-distearoylglycerol (PEG-DSG), PEG-diacylglycerol (PEG-DAG, e.g., PEG-DMG, PEG-DPG, and PEG-DSG), PEG-ceramide, PEG-distearoyl-glycero-phosphoglycerol (PEG-DSPG), PEG-dioleoyl-glycero-phosphoethanolamine (PEG-DOPE), 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, or a PEG-distearoyl-phosphatidylethanolamine (PEG-DSPE) lipid.

162. The method of claims 138 to 160, wherein the free PEG-lipid comprises a diacylphosphatidylethanolamines comprising dimyristoyl (C14) chain, Dipalmitoyl (C16) chain or Distearoyl (C18) chain.

163. The method of any one of claims 138 to 162, wherein the free PEG-lipid is present in the lipid blend in a range of 0.5-2.5 mole percent.

164. The method of any one of claims 138 to 163, wherein the free PEG-lipid comprises the same or a different lipid as the lipid in the lipid-immune cell targeting group conjugate.

165. The method of claims 138 to 164, wherein the LNP has a mean diameter in the range of 50-200 nm.

166. The method of claim 165, where the LNP has a mean diameter of about 100 nm.

167. The method of claims 138 to 166, wherein the LNP has a polydispersity index in a range from 0.05 to 1.

168. The method of claims 138 to 167, wherein the LNP has a zeta potential of from about +10 mV to about + 30 mV at pH 5.

169. The method of claims 138 to 168, wherein the nucleic acid is DNA or RNA.

170. The method of claim 169, wherein the RNA is an mRNA, tRNA, siRNA, gNRA, or microRNA.

171. The method of claim 170, wherein the mRNA encodes a receptor, a growth factor, a hormone, a cytokine, an antibody, an antigen, an enzyme, or a vaccine.

172. The method of claim 170, wherein the mRNA encodes a polypeptide capable of regulating immune response in the immune cell.

173. The method of claim 170, wherein the mRNA encodes a polypeptide capable of reprogramming the immune cell.

174. The method of claim 170, wherein the mRNA encodes a synthetic T cell receptor (synTCR) or a Chimeric Antigen Receptor (CAR).

175. The method of any one of claims 138 to 174, wherein the immune cell targeting group comprises an antibody, and the antibody is a Fab or an immunoglobulin single variable domain.

176. The method of any one of claims 138 to 174, wherein the immune cell targeting group comprises an antibody fragment selected from the group consisting of a Fab, F(ab')2, Fab'-SH, Fv, and scFv fragment.

177. The method of claim 175 or 176, wherein the immune cell targeting group comprises a Fab that comprises one or more interchain disulfide bonds.

178. The method of claim 177, wherein the Fab comprises a heavy chain fragment that comprises F174C and C233S substitutions, and a light chain fragment that comprises S176C and C214S substitutions, numbering according to Kabat.

179. The method of any one of claims 175 to 178, wherein the immune cell targeting group comprises a Fab that comprises a cysteine at the C-terminus of the heavy or light chain fragment.

180. The method of claim 175, wherein the Fab further comprises one or more amino acids between the heavy chain fragment of the Fab and the C-terminal cysteine.

181. The method of any one of claims 176 to 180, wherein the Fab comprises a heavy chain variable domain linked to an antibody CH1 domain and a light chain variable domain linked to an antibody light chain constant domain, wherein the CH1 domain and the light chain constant domain are linked by one or more interchain disulfide bonds, and wherein the immune cell targeting group further comprises a single chain variable fragment (scFv) linked to the C-terminus of the light chain constant domain by an amino acid linker.

182. The method of claim 175, wherein the immune cell targeting group comprises an immunoglobulin single variable domain.

183. The method of claim 175 or 182, wherein the immunoglobulin single variable domain comprises a cysteine at the C-terminus.

184. The method of claim 183, wherein the immunoglobulin single variable domain comprises a V_{HH} domain and further comprises a spacer comprising one or more amino acids between the V_{HH} domain and the C-terminal cysteine.

185. The method of any one of claims 175 and 182 to 184, wherein the immune cell targeting group comprises two or more V_{HH} domains.

186. The method of claim 185, wherein the two or more V_{HH} domains are linked by an amino acid linker.

187. The method of claim 185, wherein the immune cell targeting group comprises a first V_{HH} domain linked to an antibody CH1 domain and a second V_{HH} domain linked to an antibody light chain constant domain, and wherein the antibody CH1 domain and the antibody light chain constant domain are linked by one or more disulfide bonds.

188. The method of any one of claims 175, and 182 to 184, wherein the immune cell targeting group comprises a V_{HH} domain linked to an antibody CH1 domain, and wherein the antibody CH1 domain is linked to an antibody light chain constant domain by one or more disulfide bonds.

189. The method of claim 185 or 186, wherein the CH1 domain comprises F174C and C233S substitutions, and the light chain constant domain comprises S176C and C214S substitutions, numbering according to Kabat.

190. The method of any one of claims 138 to 174, wherein the immune cell targeting group comprises a Fab that comprises:
(a) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 1 and a light chain fragment comprising the amino acid sequence of SEQ ID NO:2 or 3; (b) a heavy chain fragment comprising the amino acid sequence of SEQ ID NO: 6 and a light chain fragment comprising the amino acid sequence of SEQ ID NO: 7.

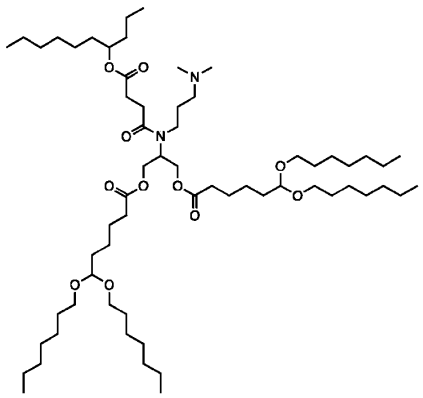
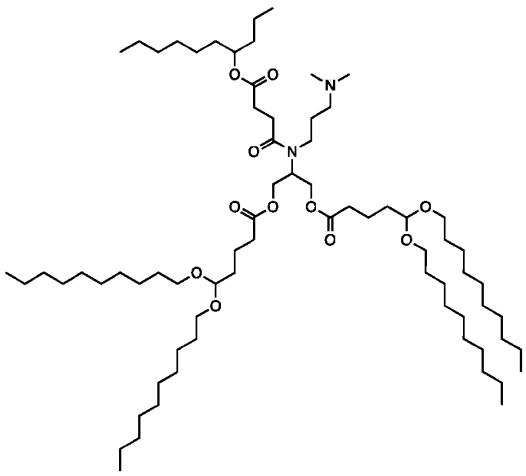
191. The method of any one of claims 138 to 190, wherein no more than 5% non-immune cells are transfected by the LNP.

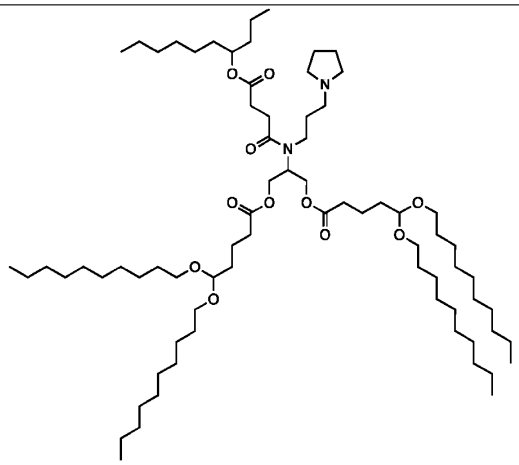
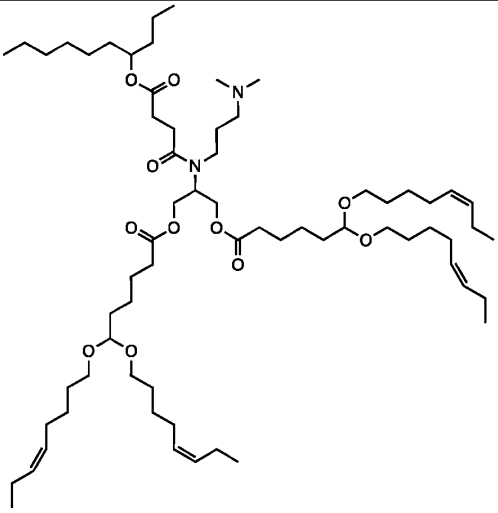
192. The method of any one of claims 138 to 191, wherein half-life of the nucleic acid delivered by the LNP or a polypeptide encoded by the nucleic acid delivered by the LNP is at least 10% longer than half-life of nucleic acid delivered by a reference LNP or a polypeptide encoded by the nucleic acid delivered by the reference LNP.

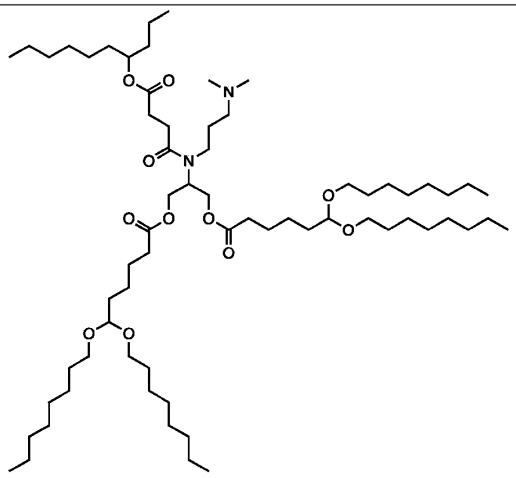
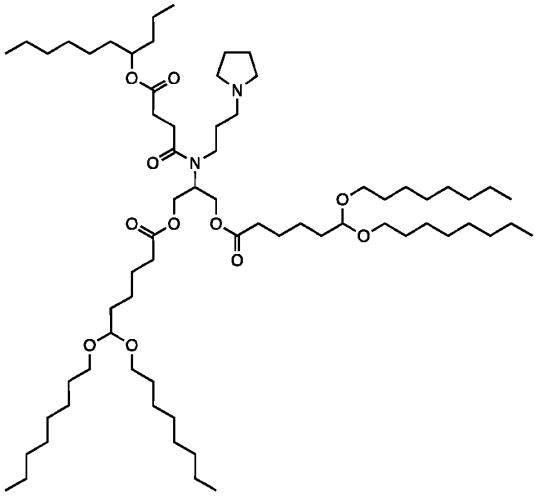
193. The method of any one of claims 138 to 192, wherein at least 10% immune cells are transfected by the LNP.

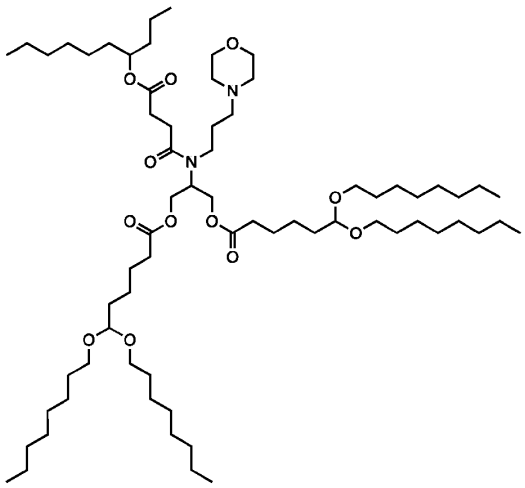
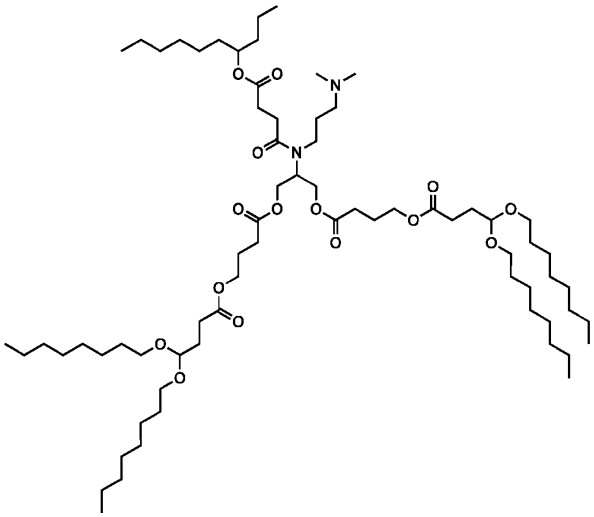
194. The method of any one of claims 138 to 193, wherein expression level of the nucleic acid delivered by the LNP is at least 10% higher than expression level of nucleic acid delivered by a reference LNP.

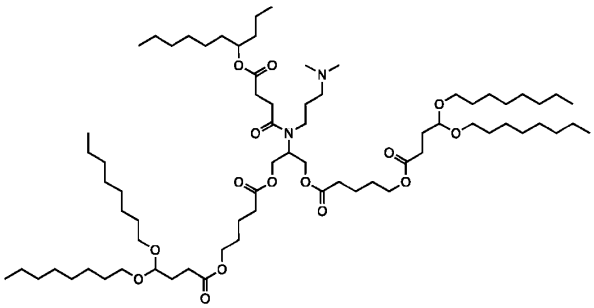
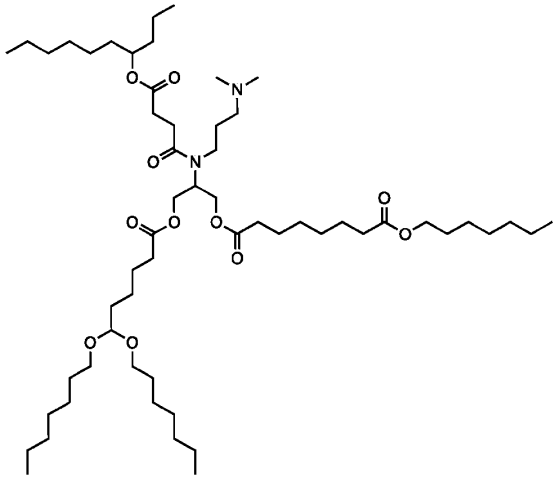
195. A compound having one of the following structures:

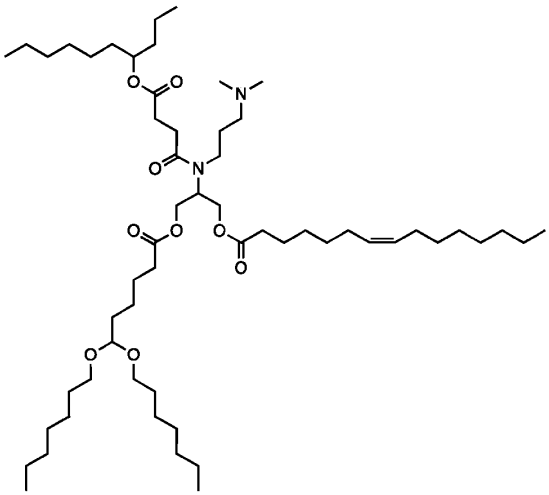
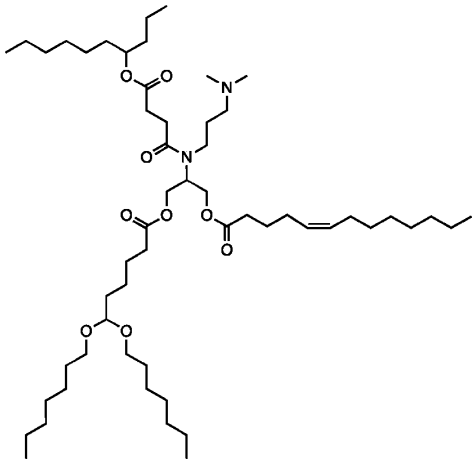
Compound	Structure	Name
A-1		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(heptyloxy)hexanoate)
A-2		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

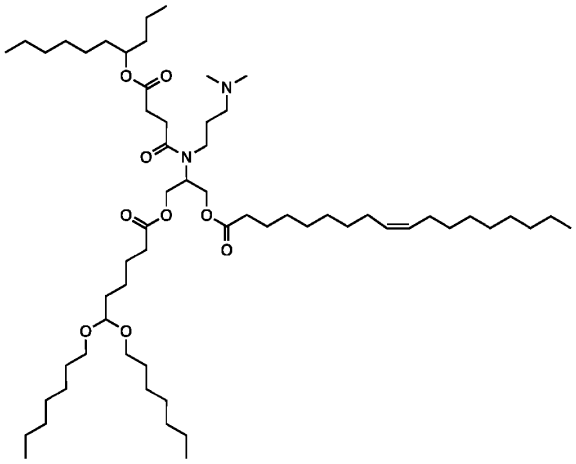
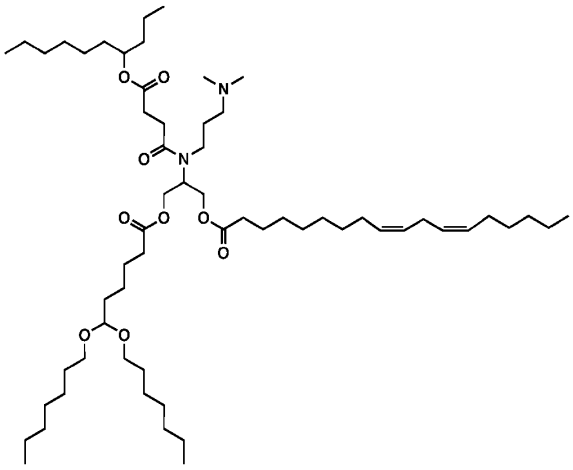
A-3		2-(4-(decan-4-yloxy)-4-oxo-N-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-4		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)

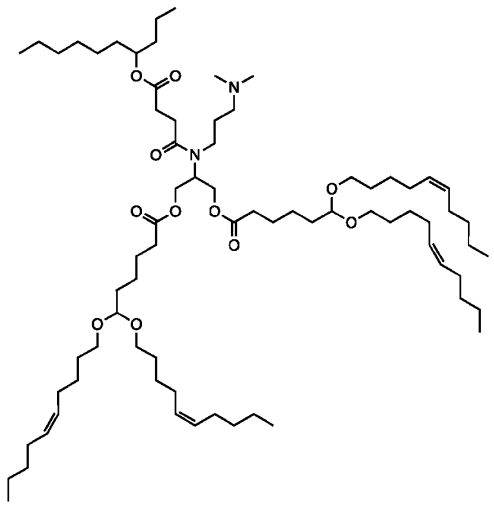
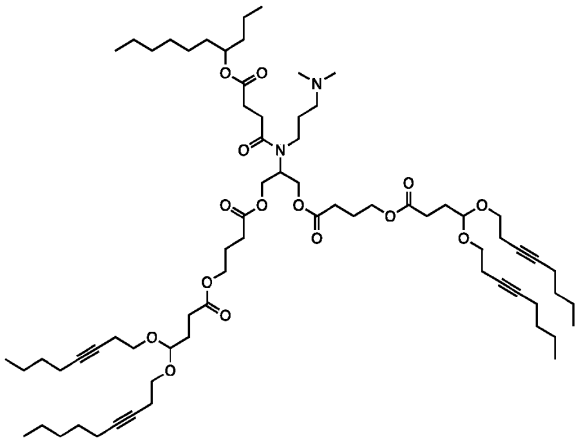
A-5		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-6		2-(4-(decan-4-yloxy)-4-oxo-N-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)

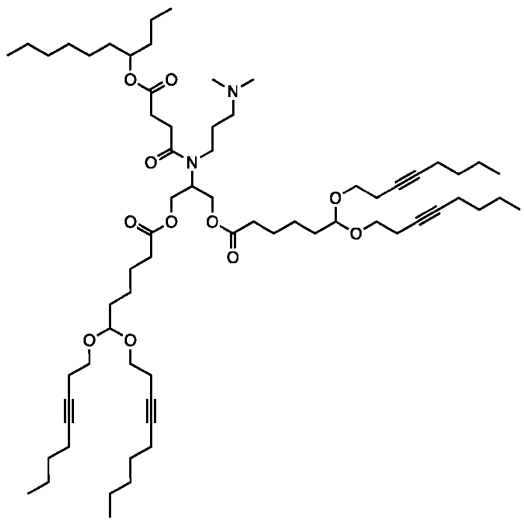
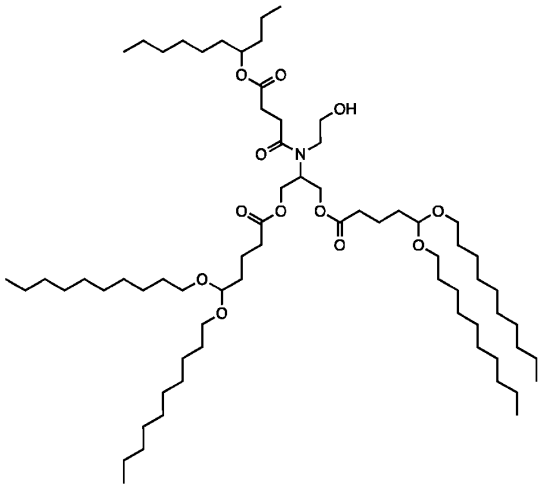
A-7		2-(4-(decan-4-yloxy)-N-(3-morpholinopropyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-8		((2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(4,4-bis(octyloxy)butanoate)

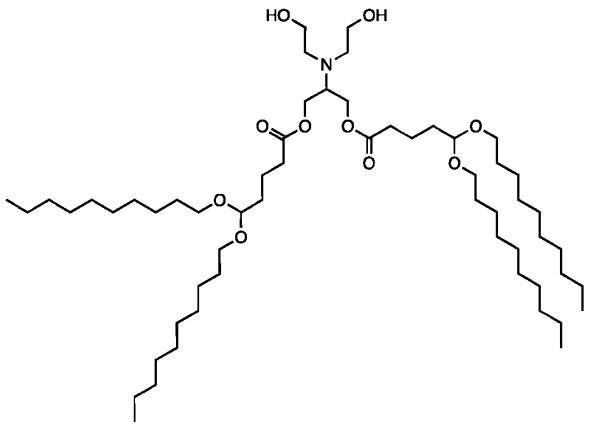
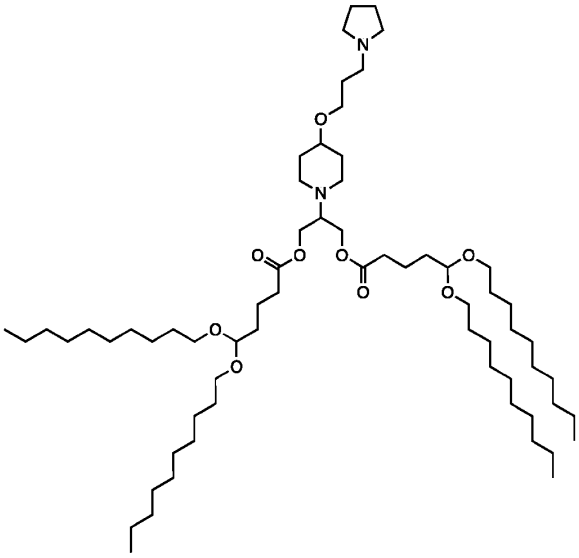
A-9		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(5-((4,4-bis(octyloxy)butanoyl)oxy)pentanoate)
A-10		1-(3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl) 8-heptyl octanedioate

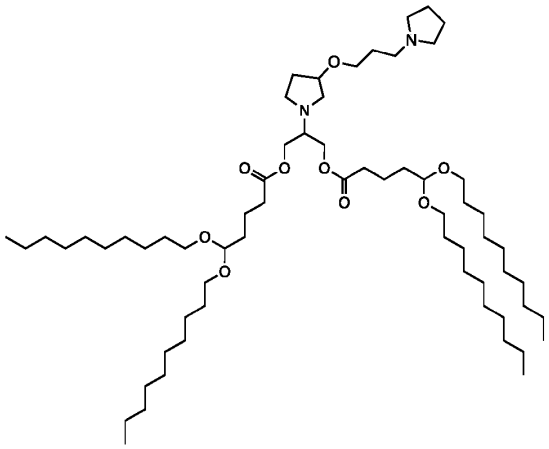
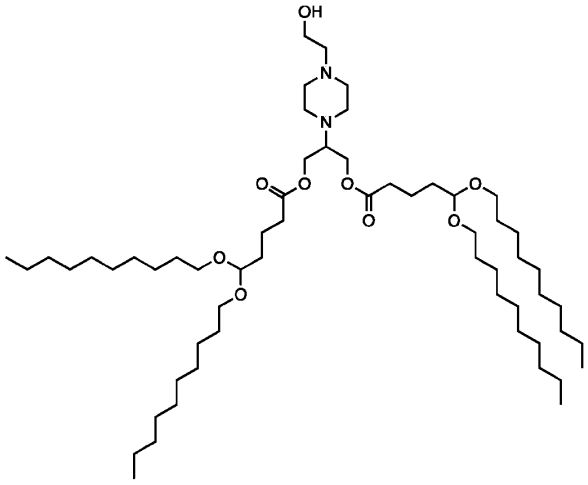
A-11		3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl (Z)-pentadec-7-enoate
A-12		3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl (Z)-tridec-5-enoate

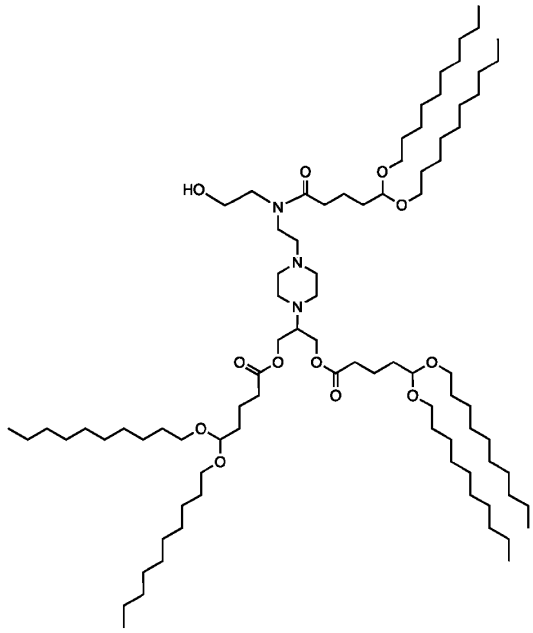
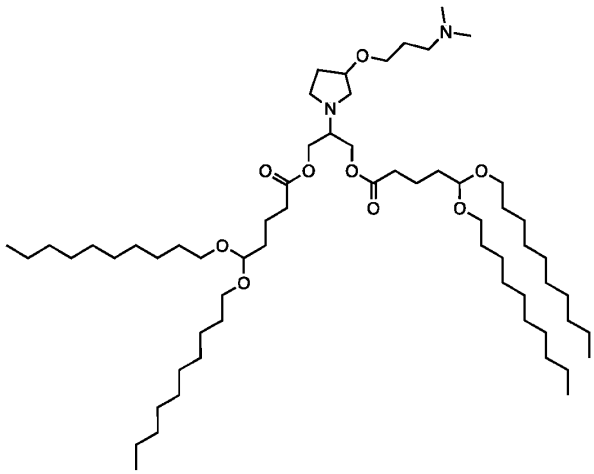
A-13		3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl oleate
A-14		3-((6,6-bis(heptyloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl (9Z,12Z)-octadeca-9,12-dienoate

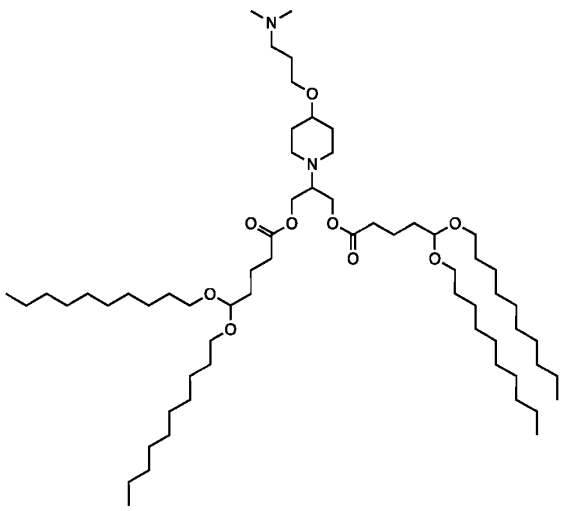
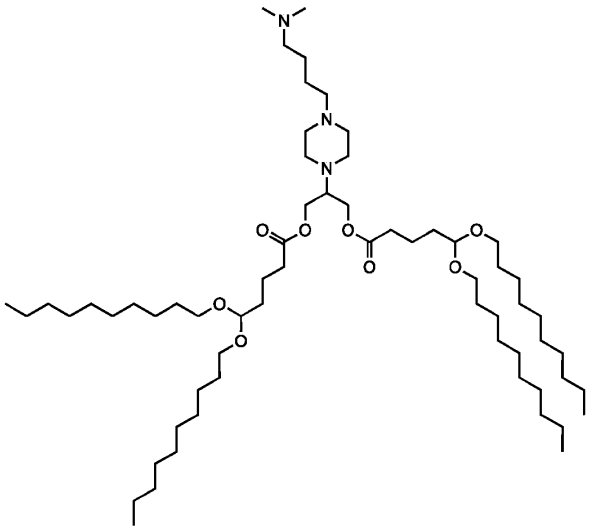
A-15		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((Z)-dec-5-en-1-yl)oxy)hexanoate)
A-16		decan-4-yl 4-((10,32-bis(oct-3-yn-1-yloxy)-13,18,24,29-tetraoxo-9,14,19,23,28,33-hexaoxadotetraconta-5,36-diyn-21-yl)(3-(dimethylamino)propyl)amino)-4-oxobutanoate

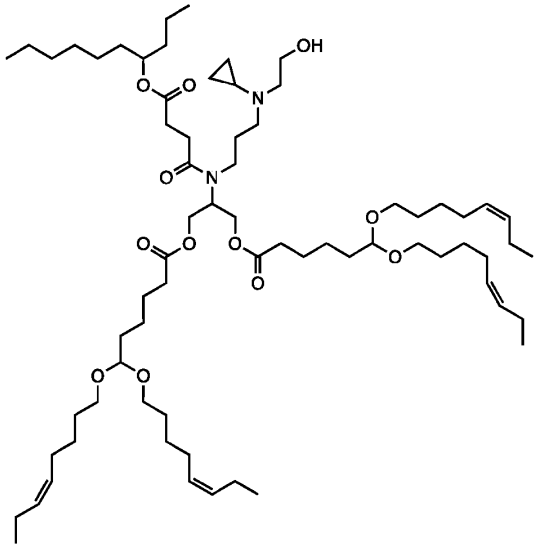
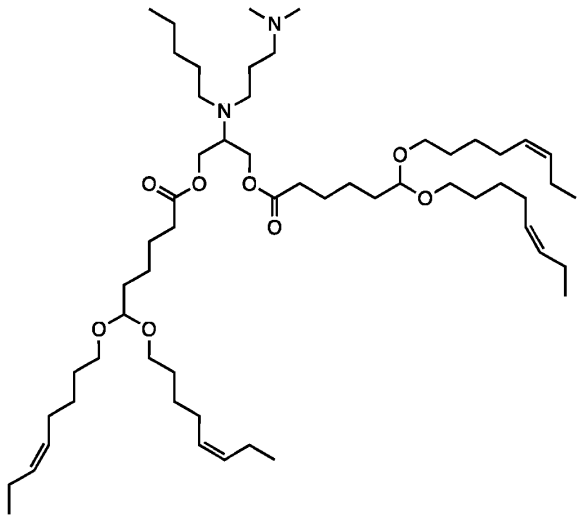
A-17		3-((6,6-bis(oct-3-yn-1-yloxy)hexanoyl)oxy)-2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propyl 6-(non-3-yn-1-yloxy)-6-(oct-3-yn-1-yloxy)hexanoate
A-18		2-(4-(decan-4-yloxy)-N-(2-hydroxyethyl)-4-oxobutanamido)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

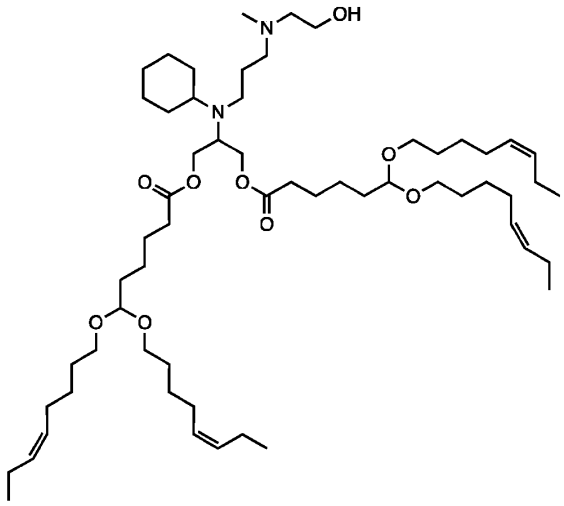
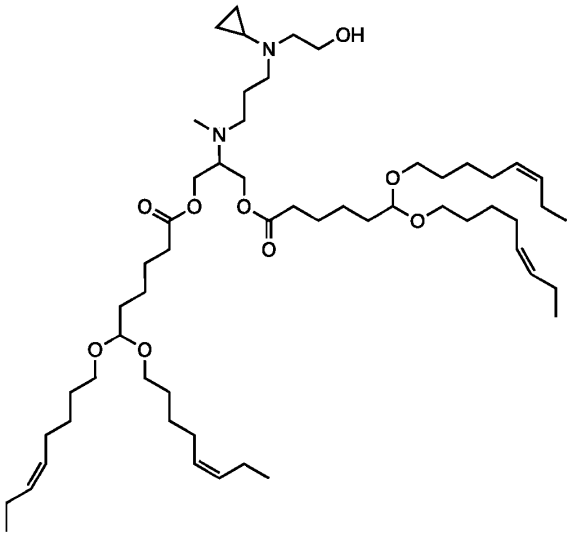
A-19		2-(bis(2-hydroxyethyl)amino)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-20		2-(4-(3-(pyrrolidin-1-yl)propoxy)piperidin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

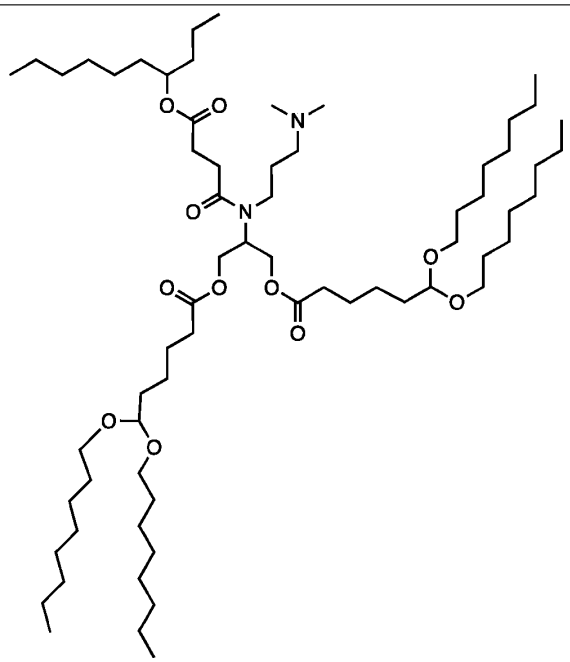
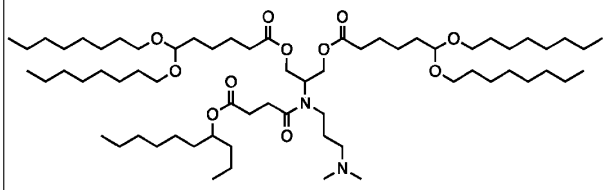
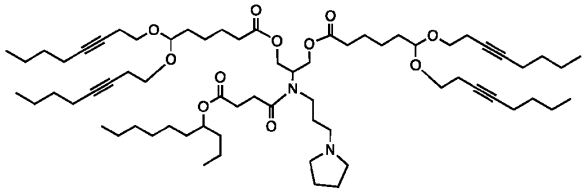
A-21		2-(3-(3-(pyrrolidin-1-yl)propoxy)pyrrolidin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-22		2-(4-(2-hydroxyethyl)piperazin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

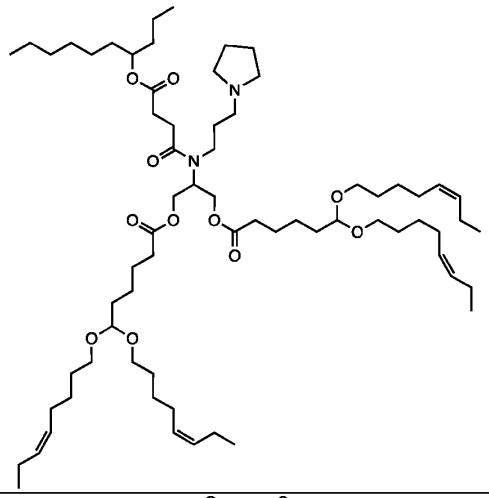
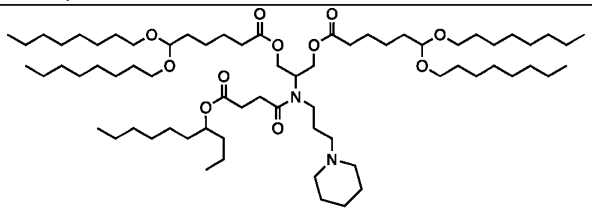
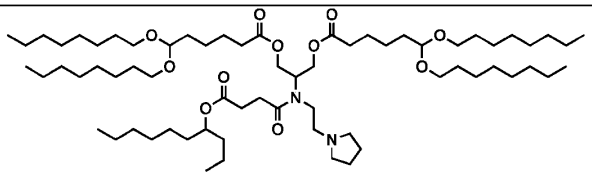
A-23		2-(4-(2-(5,5-bis(decyloxy)-N-(2-hydroxyethyl)pentanamido)ethyl)piperazin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-24-1		2-(3-(3-(dimethylamino)propoxy)pyrrolidin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

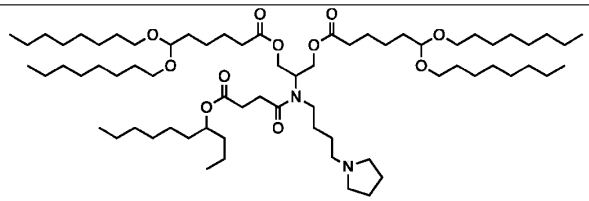
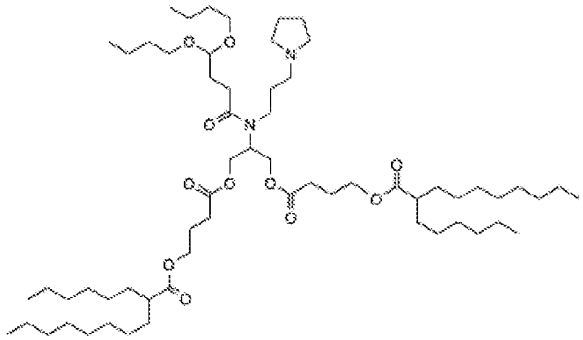
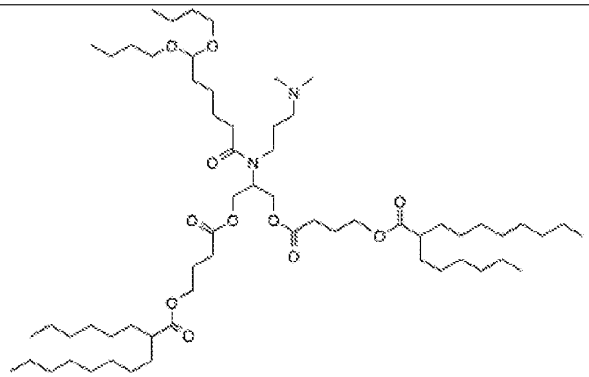
A-24		2-(4-(3-(dimethylamino)propoxy)piperidin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)
A-25		2-(4-(4-(dimethylamino)butyl)piperazin-1-yl)propane-1,3-diyl bis(5,5-bis(decyloxy)pentanoate)

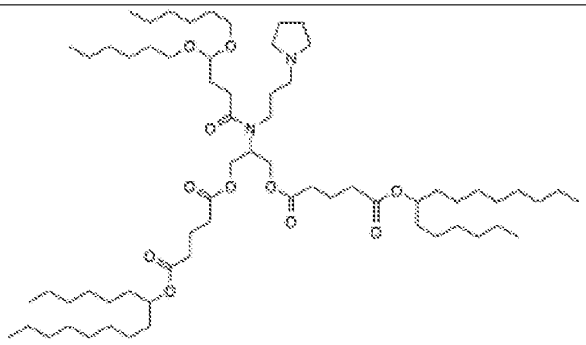
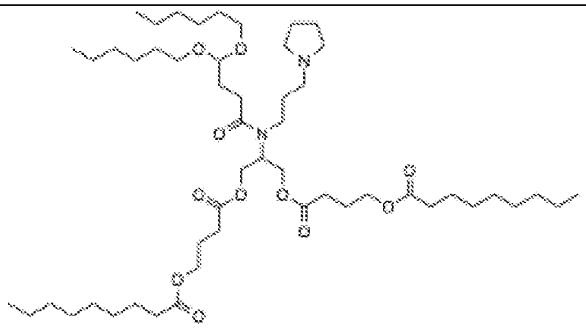
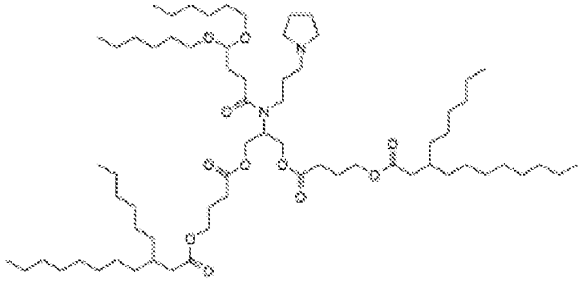
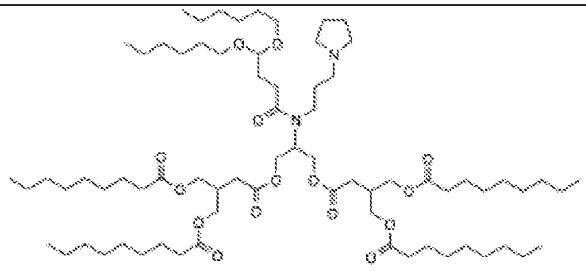
A-26		2-(N-(3-(cyclopropyl(2-hydroxyethyl)amino)propyl)-4-(decan-4-yloxy)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)
A-27		2-((3-(dimethylamino)propyl)(pentyl)amino)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)

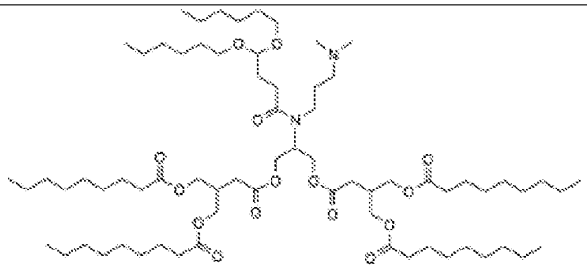
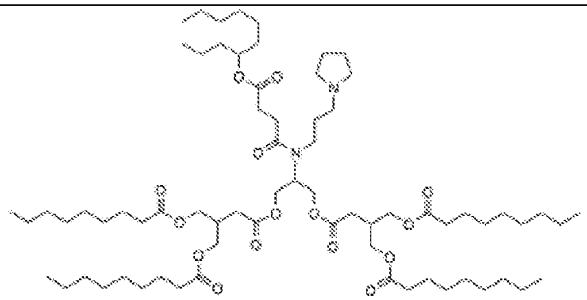
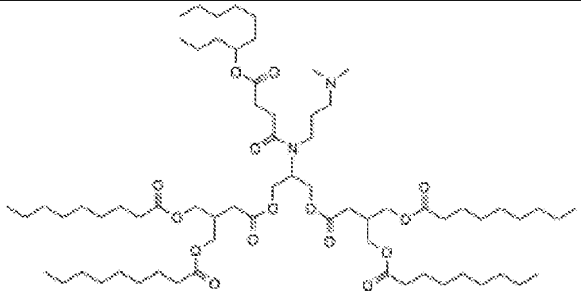
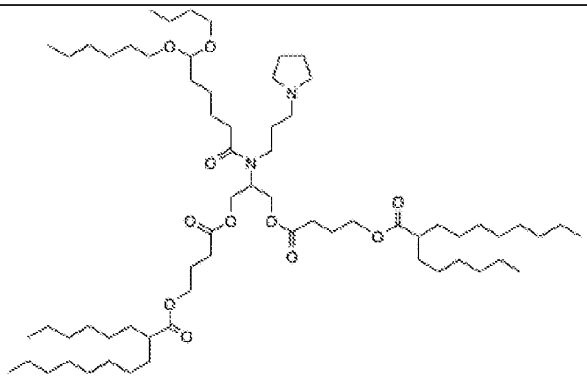
A-28		2-(cyclohexyl(3-((2-hydroxyethyl)(methyl)amino)propyl)amino)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)
A-29		2-((3-(cyclopropyl(2-hydroxyethyl)amino)propyl)(methyl)amino)propane-1,3-diyl bis(6,6-bis(((Z)-oct-5-en-1-yl)oxy)hexanoate)

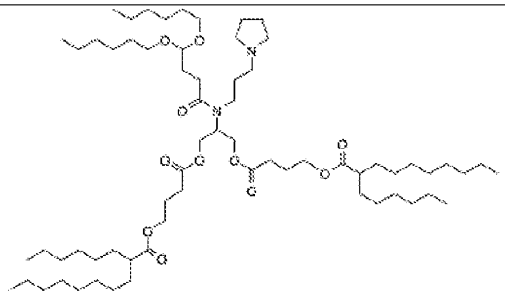
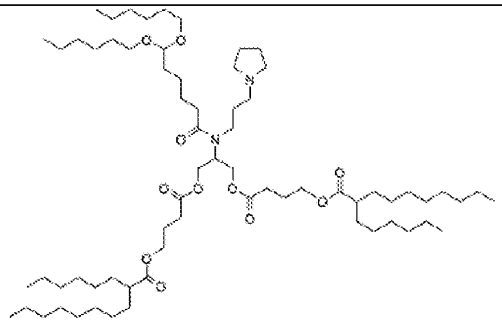
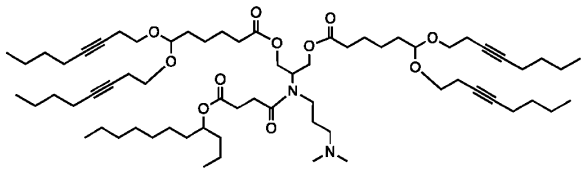
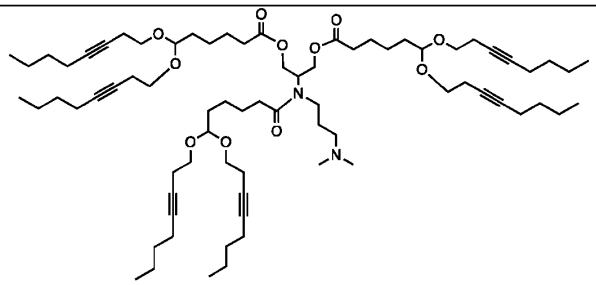
A-30		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-31		2-(4-(decan-4-yloxy)-N-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-32		2-(4-(decan-4-yloxy)-4-oxo-N-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)

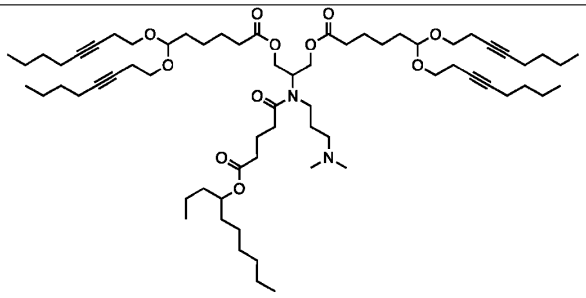
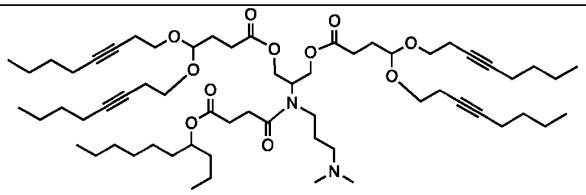
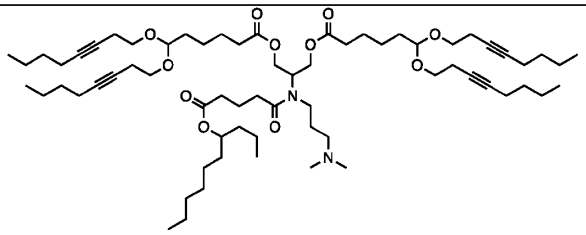
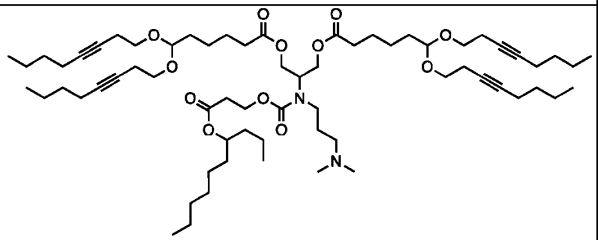
A-33		2-(4-(decan-4-yloxy)-4-oxo-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl bis(6,6-bis(((*Z*)-oct-5-en-1-yl)oxy)hexanoate)
A-34		2-(4-(decan-4-yloxy)-4-oxo-*N*-(3-(piperidin-1-yl)propyl)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-35		2-(4-(decan-4-yloxy)-4-oxo-*N*-(2-(pyrrolidin-1-yl)ethyl)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)

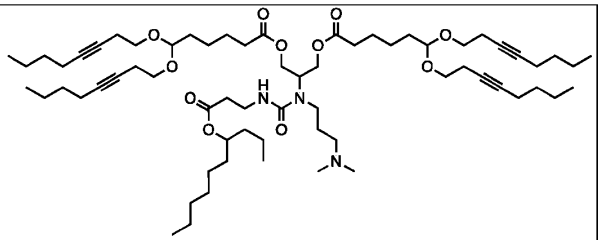
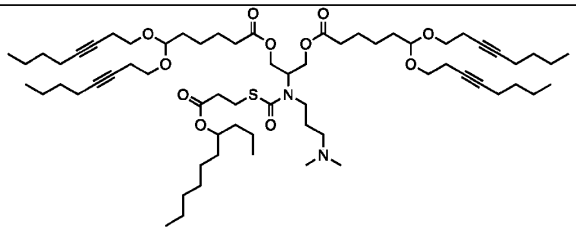
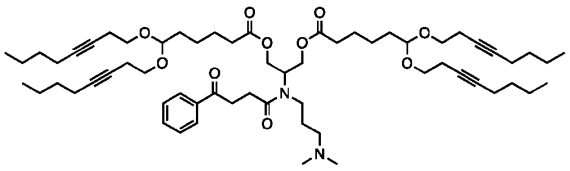
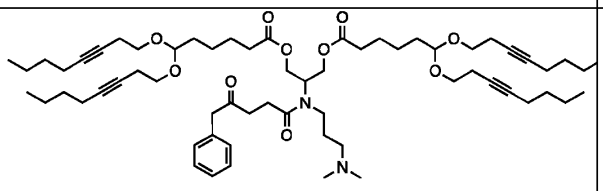
A-36		2-(4-(decan-4-yloxy)-4-oxo-*N*-(4-(pyrrolidin-1-yl)butyl)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-40		((2-(4,4-dibutoxy-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(2-hexyldecanoate)
A-41		((2-(6,6-dibutoxy-*N*-(3-(dimethylamino)propyl)hexanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(2-hexyldecanoate)

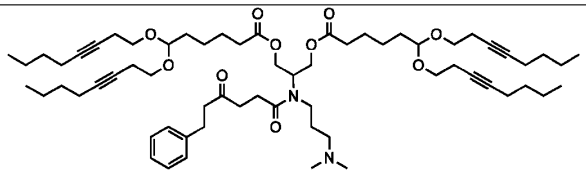
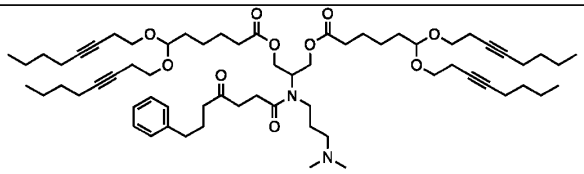
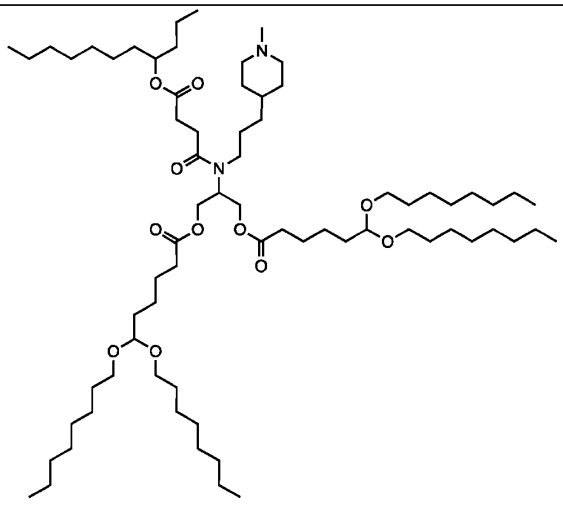
A-42		*O*,*O*'-(2-(4,4-bis(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl) di(pentadecan-7-yl) diglutarate
A-43		((2-(4,4-bis(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) dinonanoate
A-44		((2-(4,4-bis(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(3-hexylundecanoate)
A-45		(((2-(4,4-bis(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate

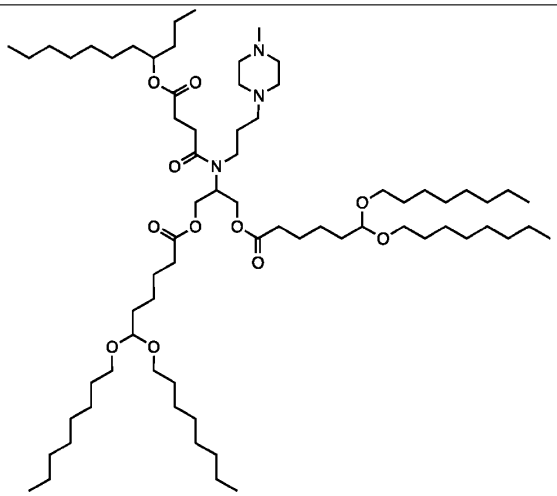
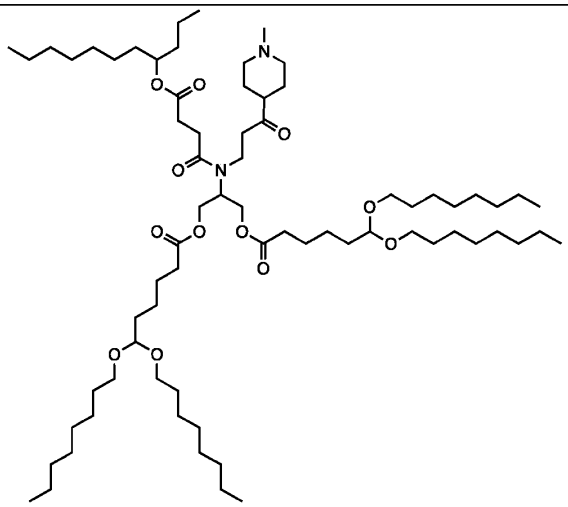
A-46		(((2-(*N*-(3-(dimethylamino)propyl)-4,4-bis(hexyloxy)butanamido)propane-1,3-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate
A-47		(((2-(4-(decan-4-yloxy)-4-oxo-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate
A-48		(((2-(4-(decan-4-yloxy)-*N*-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate
A-49		((2-(6-butoxy-6-(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)hexanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(2-hexyldecanoate)

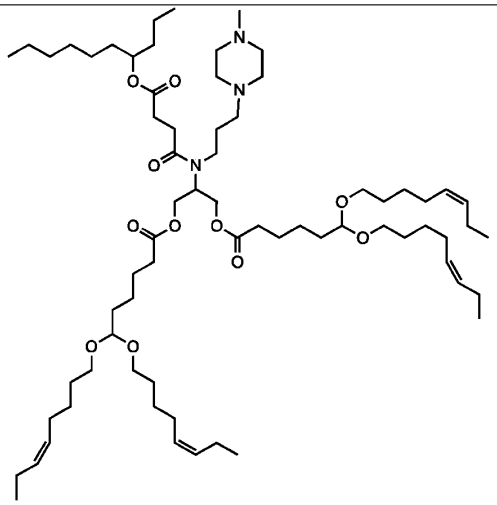
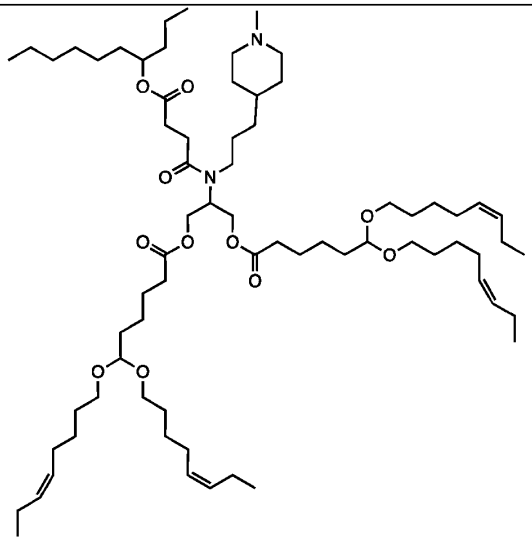
A-50		((2-(4,4-bis(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)butanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(2-hexyldecanoate)
A-51		((2-(6,6-bis(hexyloxy)-*N*-(3-(pyrrolidin-1-yl)propyl)hexanamido)propane-1,3-diyl)bis(oxy))bis(4-oxobutane-4,1-diyl) bis(2-hexyldecanoate)
A-52		2-(*N*-(3-(dimethylamino)propyl)-4-oxo-4-(undecan-4-yloxy)butanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-53		2-(*N*-(3-(dimethylamino)propyl)-6,6-bis(oct-3-yn-1-yloxy)hexanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)

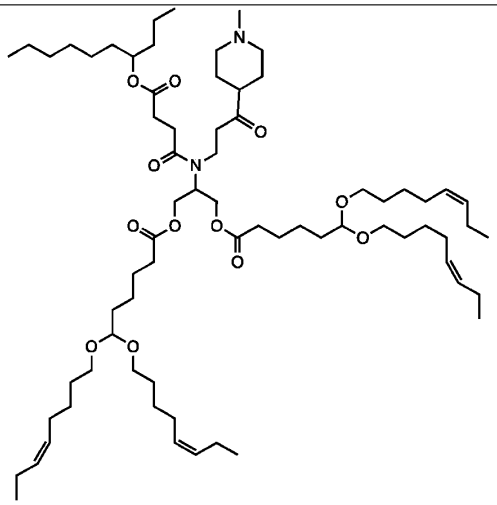
A-54		2-(5-(decan-4-yloxy)-*N*-(3-(dimethylamino)propyl)-5-oxopentanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-55		2-(4-(decan-4-yloxy)-*N*-(3-(dimethylamino)propyl)-4-oxobutanamido)propane-1,3-diyl bis(4,4-bis(oct-3-yn-1-yloxy)butanoate)
A-56		2-(5-(decan-4-yloxy)-*N*-(3-(dimethylamino)propyl)-5-oxopentanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-57		2-(((3-(decan-4-yloxy)-3-oxopropoxy)carbonyl)(3-(dimethylamino)propyl)amino)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)

A-58		2-(3-(3-(decan-4-yloxy)-3-oxopropyl)-1-(3-(dimethylamino)propyl)ureido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-59		2-((((3-(decan-4-yloxy)-3-oxopropyl)thio)carbonyl)(3-(dimethylamino)propyl)amino)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-60		2-(*N*-(3-(dimethylamino)propyl)-4-oxo-4-phenylbutanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-61		2-(*N*-(3-(dimethylamino)propyl)-4-oxo-5-phenylpentanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)

A-62		2-(*N*-(3-(dimethylamino)propyl)-4-oxo-6-phenylhexanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-63		2-(*N*-(3-(dimethylamino)propyl)-4-oxo-7-phenylheptanamido)propane-1,3-diyl bis(6,6-bis(oct-3-yn-1-yloxy)hexanoate)
A-64		2-(*N*-(3-(1-methylpiperidin-4-yl)propyl)-4-oxo-4-(undecan-4-yloxy)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)

A-65		2-(*N*-(3-(4-methylpiperazin-1-yl)propyl)-4-oxo-4-(undecan-4-yloxy)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)
A-66		2-(*N*-(3-(1-methylpiperidin-4-yl)-3-oxopropyl)-4-oxo-4-(undecan-4-yloxy)butanamido)propane-1,3-diyl bis(6,6-bis(octyloxy)hexanoate)

A-67		2-(4-(decan-4-yloxy)-*N*-(3-(4-methylpiperazin-1-yl)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((*Z*)-oct-5-en-1-yl)oxy)hexanoate)
A-68		2-(4-(decan-4-yloxy)-*N*-(3-(1-methylpiperidin-4-yl)propyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((*Z*)-oct-5-en-1-yl)oxy)hexanoate)

A-69		2-(4-(decan-4-yloxy)-*N*-(3-(1-methylpiperidin-4-yl)-3-oxopropyl)-4-oxobutanamido)propane-1,3-diyl bis(6,6-bis(((*Z*)-oct-5-en-1-yl)oxy)hexanoate)

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