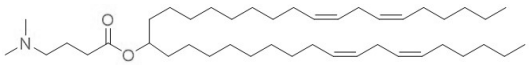
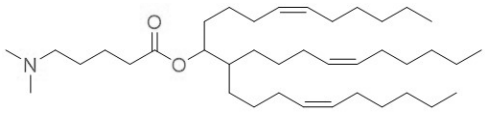
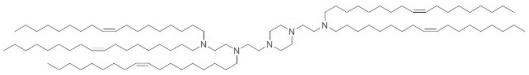
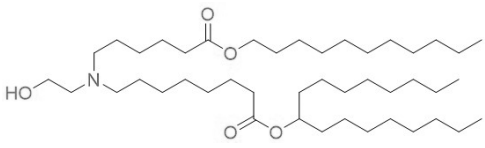
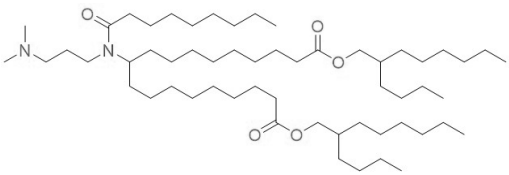
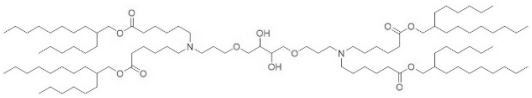
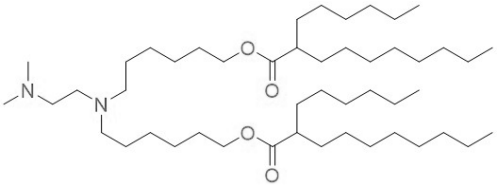
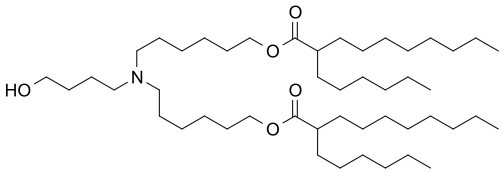
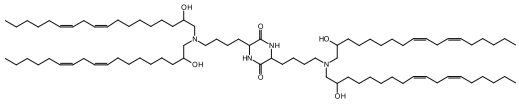
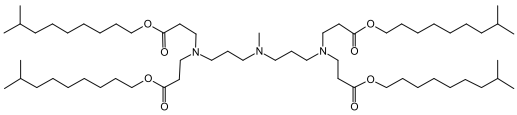
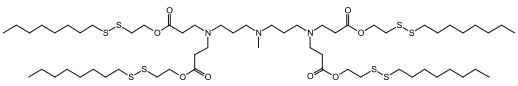
Supplementary Information for

Artificial intelligence-driven rational design of ionizable lipids for mRNA delivery

Supplementary Table Data

Supplementary Table 1. Some collected typical ionizable lipid structures and LNP formulations

ID of lipid	Chemical structure of the ionizable lipid	Formulation of the LNP	Ref.
MC3		Lipid: DSPC: Chol: DMG-PEG (mol%) =50:10:38.5:1.5	1–8
Amino Lipid 13 (LP01)		Lipid: DSPC: Chol: PEG-c-DMA (mol%) =54.6:10.9:32.8:1.6	2
Compound of formula (IV)		Lipid: DOPE: Chol: DMG-PEG (mol%) = 40:20:38.5:1.5	3
Compound 25 (SM-102)		Lipid: DSPC: Chol: DMG-PEG (mol%) =50:10:38.5:1.5	4,5
Compound 9		Lipid: DSPC: Chol: PEG-DMA (mol%) =50:10:38.5:1.5	6

Compound 2		Lipid: DSPC: Chol: PEG-DMA (mol%) =50:10:38.5:1.5	7
Compound 5		Lipid: DSPC: Chol: PEG-DMA (mol%) =50:10:38.5:1.5	8
Compound 3 (ALC-0315)		Lipid: DSPC: Chol: PEG-DMA (mol%) =50:10:38.5:1.5	1
OF-02		Lipid: DOPE: Chol: C14-PEG (mol%) = 35:16:46.5:2.5	9
306Oi10		Lipid: DOPE: Chol: C14-PEG (mol%) = 35:16:46.5:2.5	10
306-O12B		Lipid: Chol: DOPC: DMG-PEG (mol%) =50:38.5:10:1.5	11

Supplementary Table 2. Performance of the regression model predicting mRNA delivery efficiency

Data source	Training set						Test set		
	MAE	MSE	R^2	MAE_5CV	MSE_5CV	R^2_5CV	MAE	MSE	R^2
Moderna*	0.38	0.25	0.89	0.86	1.21	0.43	0.80	0.97	0.52
Acuitas & Protiva*	0.09	0.01	0.98	0.32	0.23	0.73	0.28	0.18	0.77
Combined*	0.03	0.00	0.97	0.11	0.02	0.58	0.08	0.01	0.72

*The data collected from patents were provided by three sources, Moderna, Acuitas, and Protiva company, respectively.

5_CV, 5-fold cross validation, MAE, mean absolute error; MSE, mean squared error; RMSE, root mean squared error; R^2, determination coefficient

Supplementary Table 3. Performance of classification models predicting mRNA delivery efficiency.

Model	Index	Training set	Training set 5CV	Test set
LightGBM (Model 1)	Recall	0.86	0.75	0.81
	Precision	0.88	0.79	0.76
	ACC	0.90	0.82	0.82
	F1	0.87	0.76	0.78
Random Forest	Recall	0.84	0.76	0.74
	Precision	0.90	0.76	0.77
	ACC	0.90	0.81	0.81
	F1	0.87	0.76	0.75

Source data are provided as a Source Data file. LightGBM, Light Gradient Boosting Machine; 5_CV, 5-fold cross validation; ACC, Accuracy; F1, F1_score.

Supplementary Table 4. Performance of the AI model predicting apparent pKa.

	Training set	Validation set	Test set
RMSE	0.28	0.32	0.25
MAE	0.18	0.22	0.19
R^2	0.79	0.61	0.59

MAE, mean absolute error; MSE, mean squared error; RMSE, root mean squared error; R^2, determination coefficient

Supplementary Table 5. External validation of predicting mRNA delivery efficiency

Lipid	Prediction	Prediction_all	Experiment
1	N	P	P
2	N	N	P
3	P	P	P
4	P	P	P
5	P	P	N*
6	N	P	P
7	P	N	N
8	N	N	N
9	N	N	N
10	N	P	N
11	N	N	N
12	N	N	N
13	P	N	N
14	N	N	N
Correct rate	0.57	0.78	-

*Approximates the threshold of MC3. P, positive; N, negative.

“Prediction” represents the prediction provided by the model trained on partial data, while “Prediction_all” represents the prediction of a model trained on the whole dataset after hyperparameter tuning method had been identified and fixed.

Supplementary Table 6. Characterization of LNPs containing LQ085, 086 and 087 (n=3, mean±SD)

Ionizable lipids	Particle size (nm)	PDI	EE (%)	pKa (tested)	pKa (predicted)	Probability of positive delivery efficiency
SM-102	79.243±0.952	0.109±0.011	93.870	6.574	-	-
MC3	63.470±0.426	0.118±0.003	96.370	6.214	-	-
LQ085	92.337±0.542	0.084±0.016	97.720	7.392	6.516	0.911
LQ086	79.717±1.349	0.181±0.008	96.430	7.402	6.393	0.824
LQ087	76.087±1.064	0.132±0.012	94.340	6.560	6.132	0.790

Source data are provided as a Source Data file. EE, encapsulation efficiency; PDI, polydispersity index.

Supplementary Table 7. Performance of the Model 2 predicting mRNA delivery efficiency

	Training set	Validation set_5CV	Test set
Recall	0.78	0.52	0.59
Precision	0.81	0.60	0.68
ACC	0.89	0.77	0.81
F1	0.79	0.56	0.63

Source data are provided as a Source Data file. 5_CV, 5-fold cross validation; ACC, Accuracy; F1, F1_score.

Supplementary Table 8. Performance of the Model 2 on original dataset

	Training set	Training set 5CV	Test set
Recall	0.59	0.75	0.58
Precision	0.90	0.73	0.82
ACC	0.81	0.79	0.78
F1	0.71	0.74	0.68

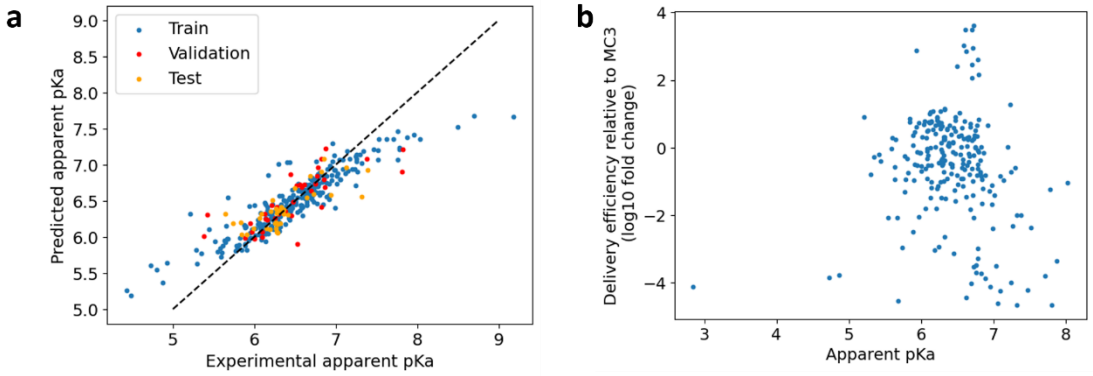
Source data are provided as a Source Data file. 5_CV, 5-fold cross validation; ACC, Accuracy; F1, F1_score.

Supplementary Table 9. Characterization of LNPs containing LQ089-094 (n=3, mean±SD)

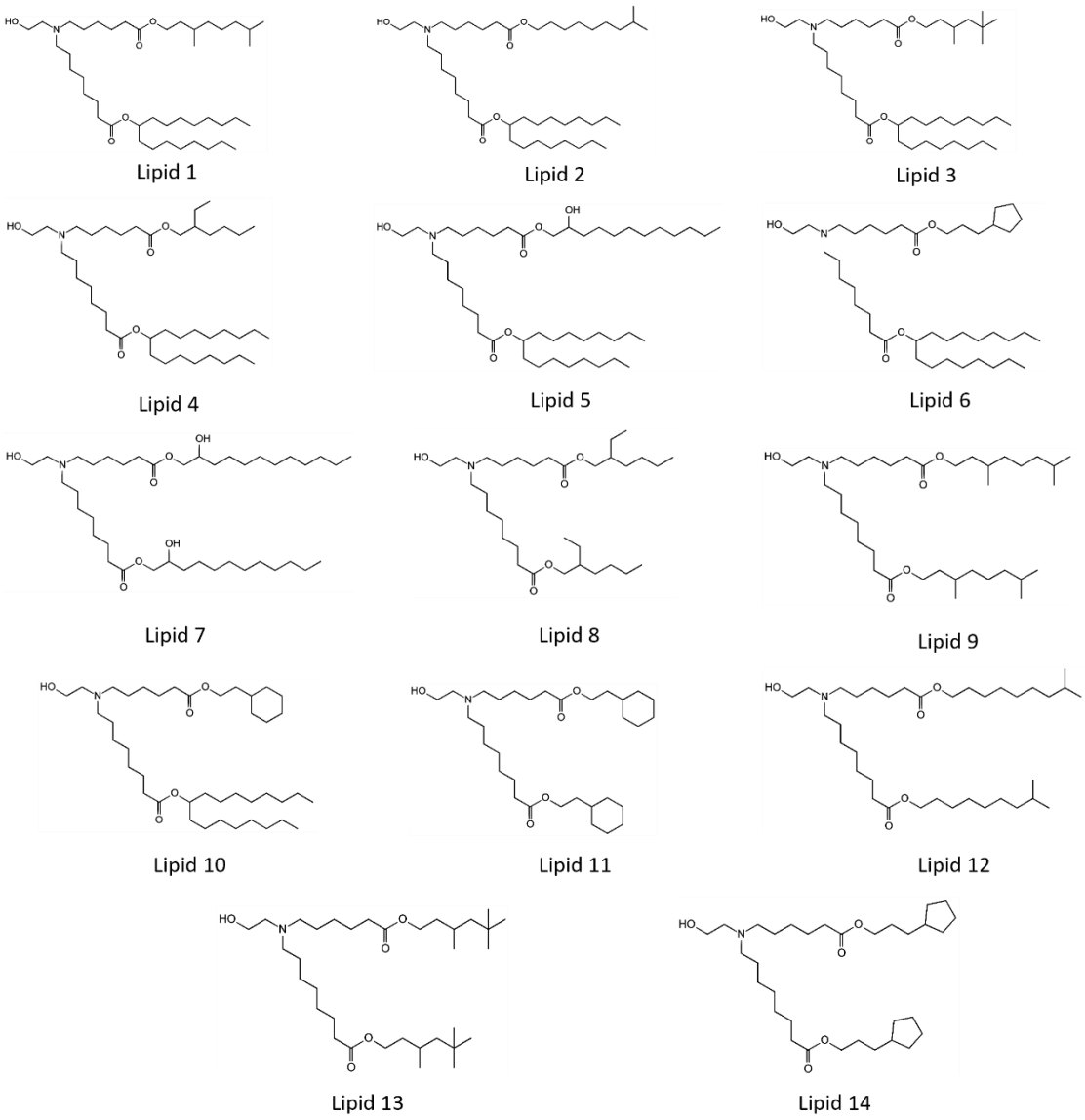
Ionizable lipids	Particle size (nm)	PDI	EE (%)	pKa (tested)	pKa (predicted)	Probability of positive delivery efficiency
SM-102	77.973±0.679	0.106±0.014	91.320	-	-	-
MC3	58.267±0.261	0.131±0.003	94.854	-	-	-
LQ089	60.900±0.095	0.111±0.004	95.650	6.540	6.676	0.514
LQ090	60.207±0.280	0.140±0.020	96.920	6.118	6.286	0.507
LQ091	50.537±0.170	0.090±0.023	96.080	6.226	6.412	0.517
LQ092	54.470±0.251	0.120±0.006	94.866	6.134	6.437	0.509
LQ093	55.593±0.284	0.094±0.005	96.207	6.115	6.569	0.520
LQ094	55.517±0.365	0.131±0.005	93.745	6.062	6.569	0.520

Source data are provided as a Source Data file. EE, encapsulation efficiency; PDI, polydispersity index.

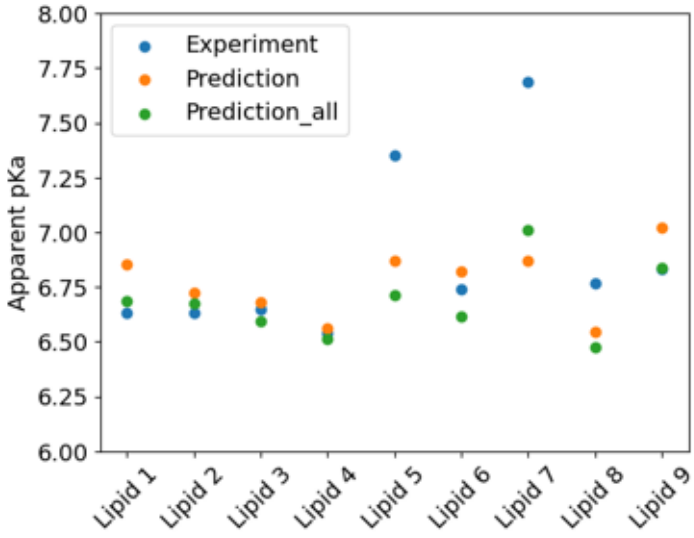
Supplementary Figure Data



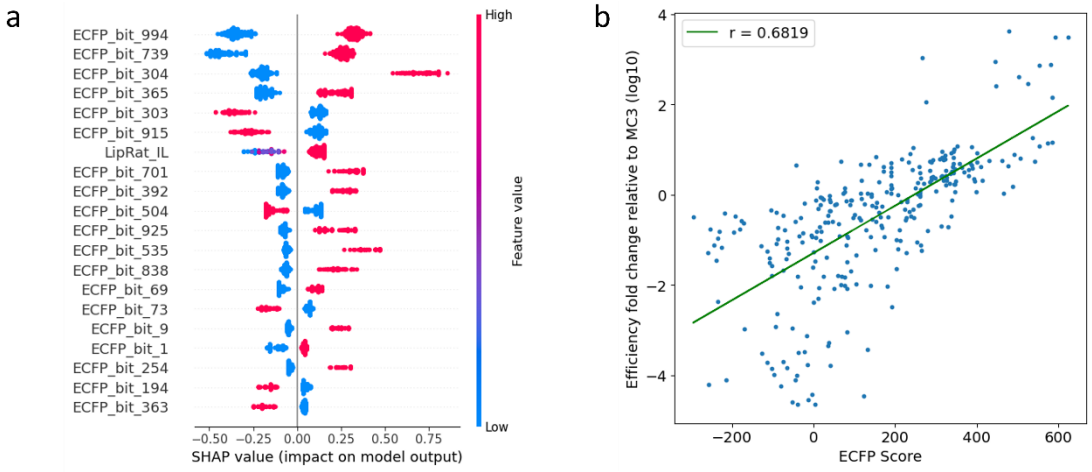
Supplementary Figure 1. Apparent pKa model is accurate in optimal pKa range. (a) Comparison between predicted and experimental pKa values. (b) Scatter plot of the fold-change in mRNA delivery efficiency relative to MC3 and the apparent pKa of LNPs in the dataset. Source data are provided as a Source Data file. MC3, DLin-MC3-DMA.



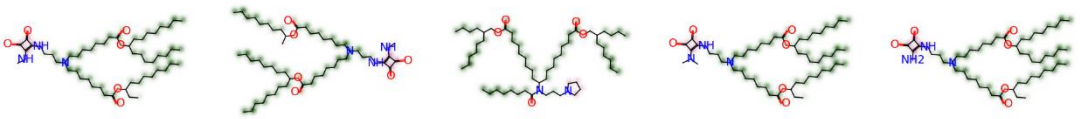
Supplementary Figure 2. Ionizable lipids used for external validation



Supplementary Figure 3. External validation of models predicting apparent pKa. Source data are provided as a Source Data file. “Prediction” represents the prediction provided by the model trained on partial data, while “Prediction_all” represents the prediction of a model trained on the whole dataset after hyperparameter tuning method had been identified and fixed.



Supplementary Figure 4. Impacts of ECFP of ionizable lipids on mRNA delivery efficiency. (a), Feature significance of the LightGBM classification model predicting the mRNA delivery efficiency. Each dot represents how the feature influences the output for one sample in the dataset. (b), The linear relationship between the ECFP scores of ionizable lipids and their fold-change in mRNA delivery efficiency relative to MC3. Source data are provided as a Source Data file. LipRat_IL, ionizable lipid molar ratio in the formulation. LightGBM, Light Gradient Boosting Machine; ECFP, extended connectivity fingerprints. MC3, DLin-MC3-DMA.



fold: 3077.5 ecfp_score: 623.6 | fold: 3042.5 ecfp_score: 592.6 | fold: 14.4 ecfp_score: 585.7 | fold: 143.8 ecfp_score: 584.3 | fold: 764.1 ecfp_score: 582.0

fold: 13.7 ecfp_score: 573.0 | fold: 12.0 ecfp_score: 553.1 | fold: 720.8 ecfp_score: 553.1 | fold: 5.5 ecfp_score: 542.5 | fold: 18.5 ecfp_score: 536.6

fold: 291.5 ecfp_score: 525.8 | fold: 6.4 ecfp_score: 506.6 | fold: 409.4 ecfp_score: 503.9 | fold: 7.8 ecfp_score: 492.6 | fold: 1.5 ecfp_score: 488.1

fold: 4140.3 ecfp_score: 478.4 | fold: 4.2 ecfp_score: 477.1 | fold: 1.1 ecfp_score: 465.4 | fold: 5.7 ecfp_score: 462.1 | fold: 252.0 ecfp_score: 446.9

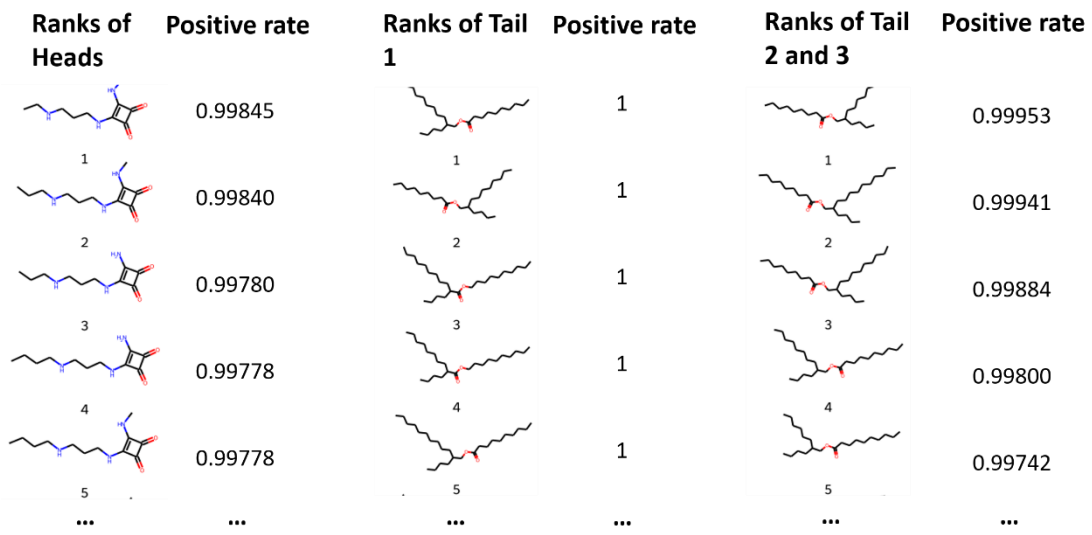
fold: 892.3 ecfp_score: 444.5 | fold: 5.6 ecfp_score: 431.1 | fold: 2.1 ecfp_score: 413.2 | fold: 4.2 ecfp_score: 409.2 | fold: 5.2 ecfp_score: 401.4

fold: 8.3 ecfp_score: 401.0 | fold: 1.2 ecfp_score: 398.3 | fold: 1.9 ecfp_score: 395.3 | fold: 2.2 ecfp_score: 393.7 | fold: 3.8 ecfp_score: 389.9

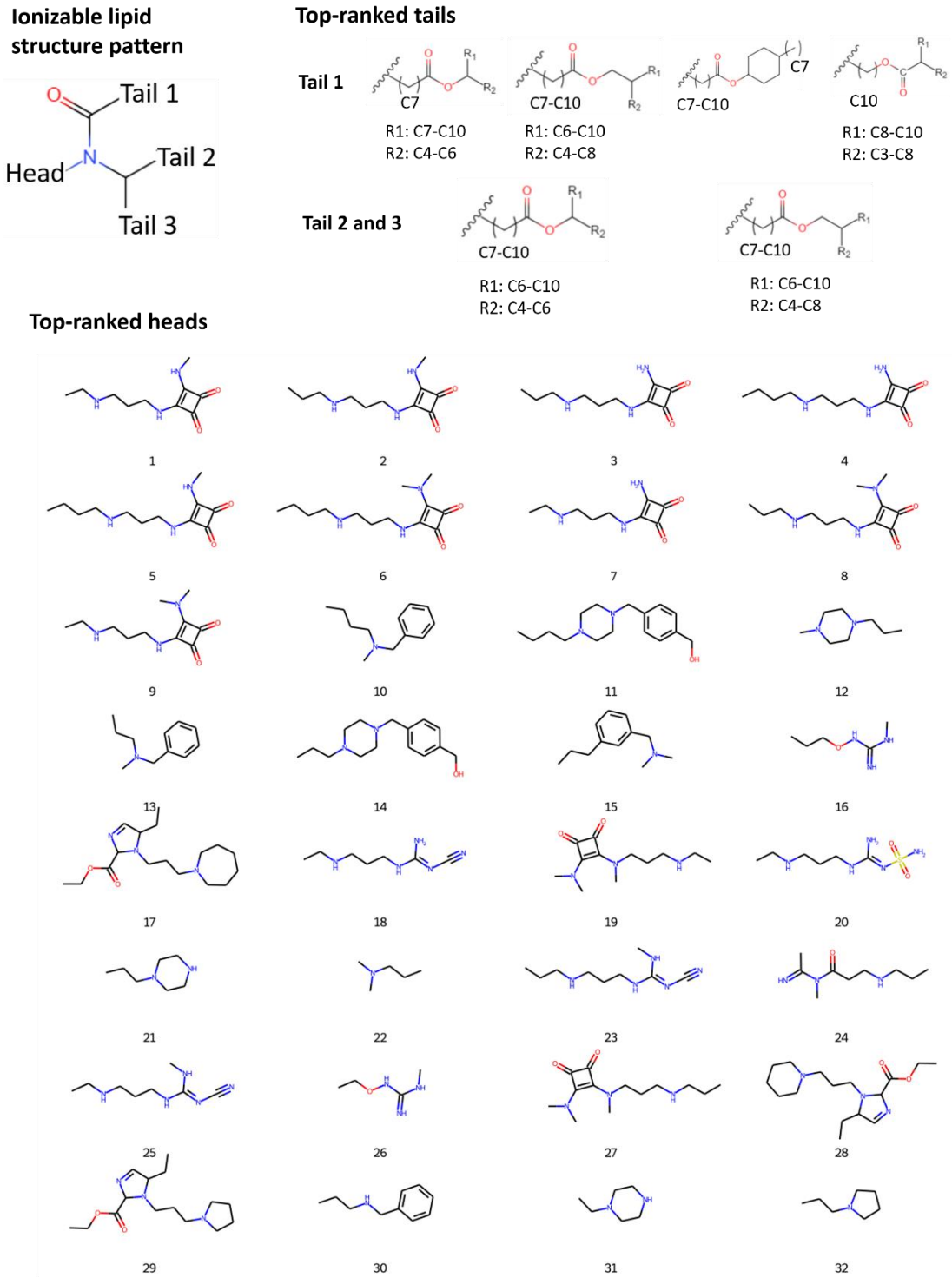
fold: 11.5 ecfp_score: 388.4 | fold: 4.6 ecfp_score: 388.4 | fold: 4.9 ecfp_score: 383.1 | fold: 2.6 ecfp_score: 380.8 | fold: 3.5 ecfp_score: 380.7

fold: 3.2 ecfp_score: 368.1 | fold: 3.9 ecfp_score: 364.1 | fold: 4.7 ecfp_score: 358.5 | fold: 12.0 ecfp_score: 352.2 | fold: 2.0 ecfp_score: 349.0

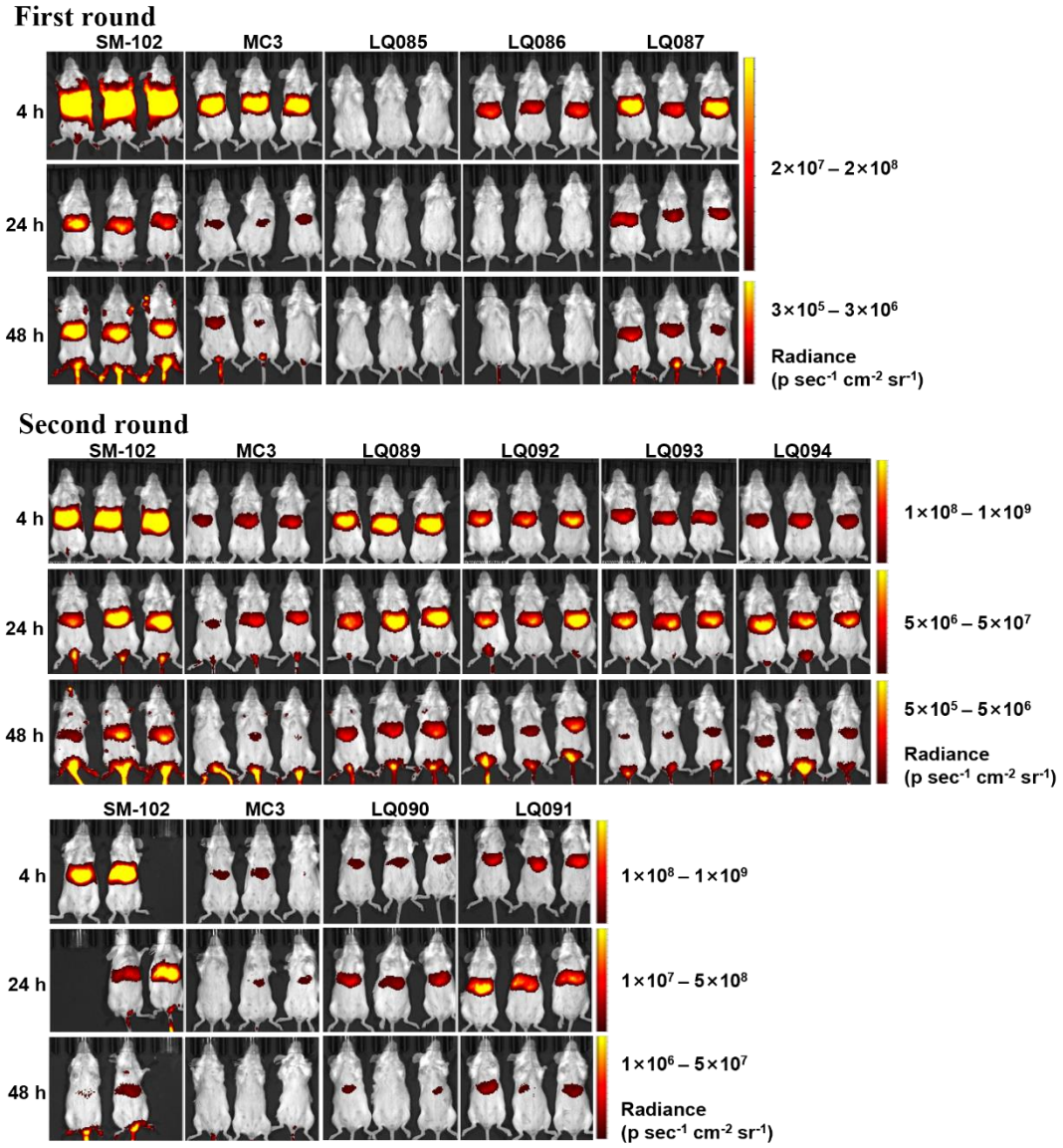
Supplementary Figure 5. Molecular similarity of top 40 ionizable lipid structures with the highest ECFP score. In each molecule, the section which is similar to other molecules in the dataset is colored in green, while the distinct section is colored in red. The fold changes relative to MC3 LNP are also shown along lipid molecules.



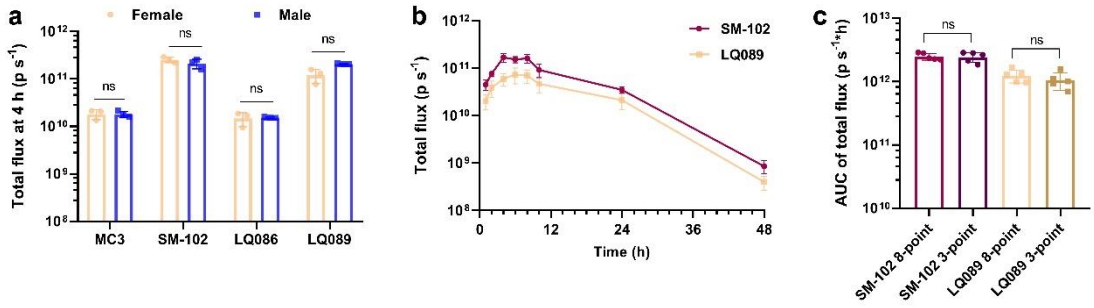
Supplementary Figure 6. Positive rates of top 5 segments constituting the ionizable lipids. Positive rate was calculated as the number of



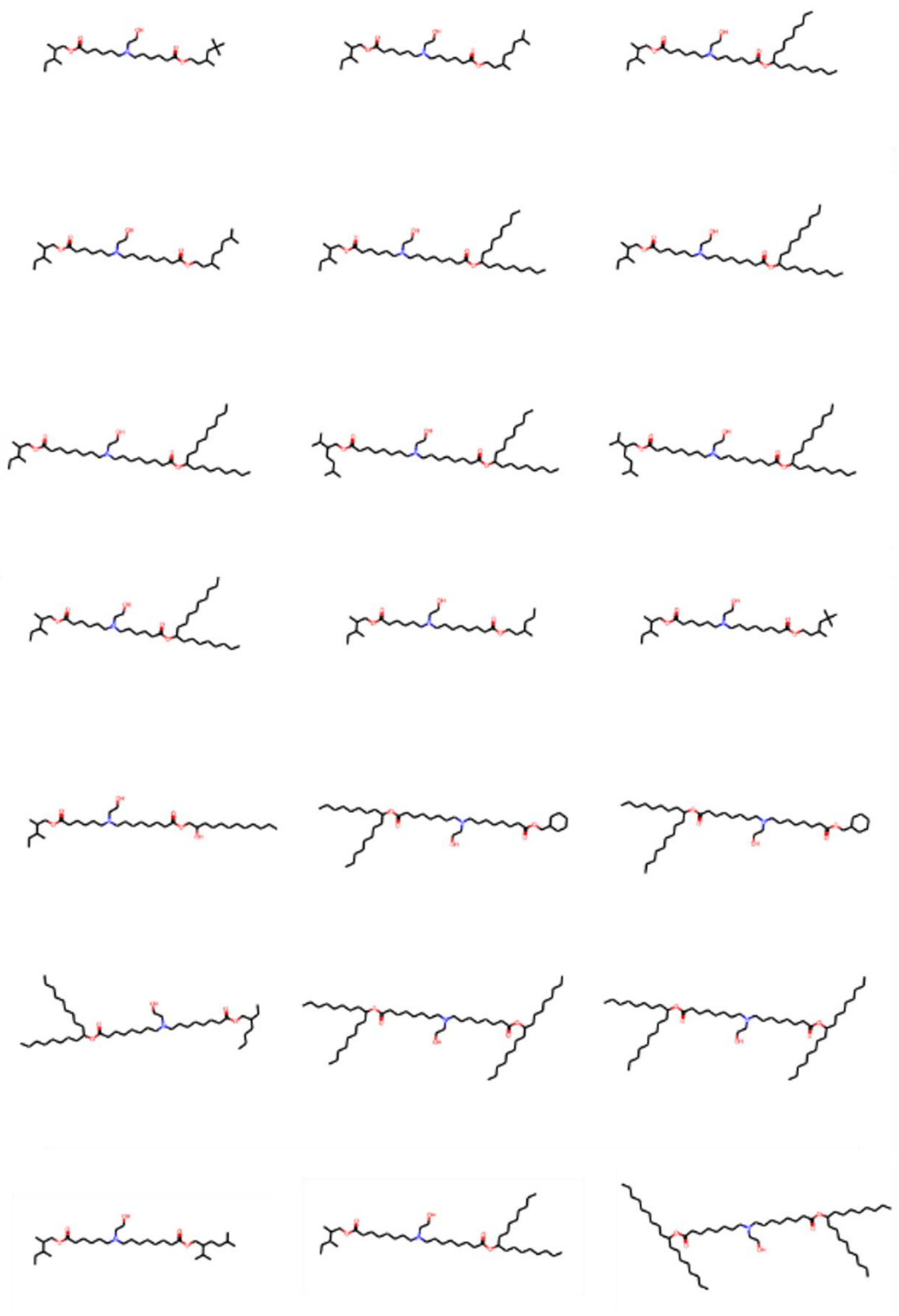
Supplementary Figure 7. Structures of ionizable lipids selected from the virtual screening and the rank of 32 best head groups.



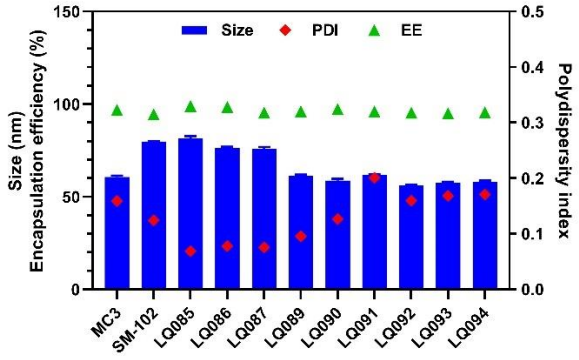
Supplementary Figure 8. Total lufiferase luminescence using the spectrum imaging system for ionizable lipids selected from the two rounds of virtual screening. MC3, DLin-MC3-DMA.



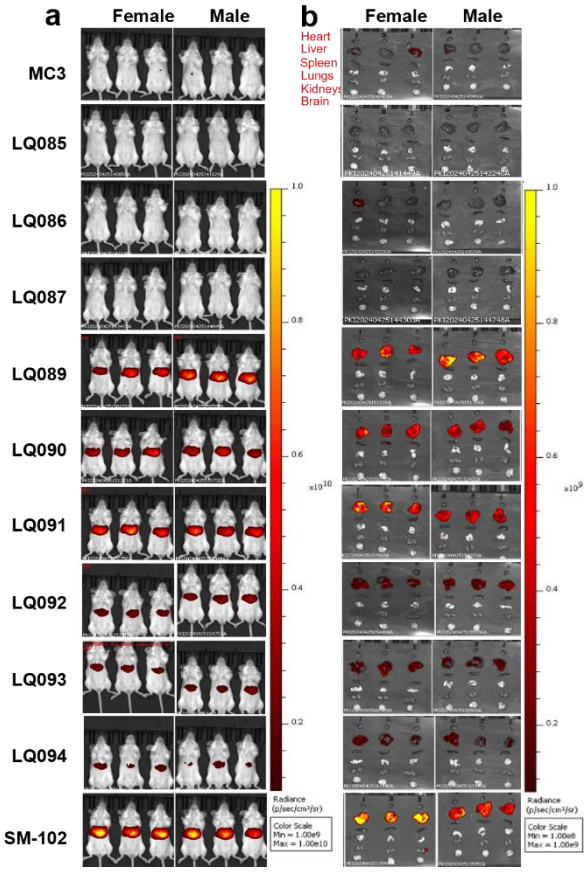
Supplementary Figure 9. Methodological validation of the method for evaluating the lipids. (a), Each LNP was test with three female BALB/c mice and three males, and the results showed no difference between the sexes. (b), Bioluminescence-time curve. Eight time points: 1, 2, 4, 6, 8, 10, 24, 48 h. (n = 5) (c), The AUC was calculated by 3-point method (4, 24, 48h) and 8-point method respectively, resulting in no difference between the two methods. Therefore, in order to evaluate new lipids in a more convenient way, fluorescence signals were monitored at 4, 24, and 48 hours post administration. Source data are provided as a Source Data file. MC3, DLin-MC3-DMA; LNP, lipid nanoparticle; AUC, area under curve.



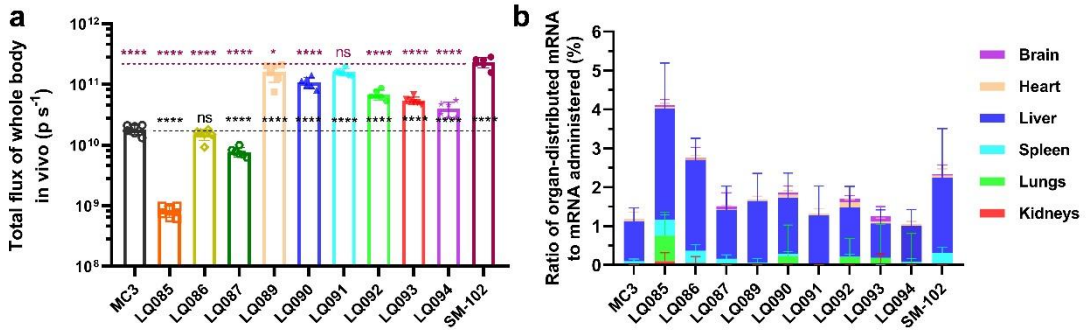
Supplementary Figure 10. 21 lipids with positively predicted mRNA delivery efficiency from Model 2



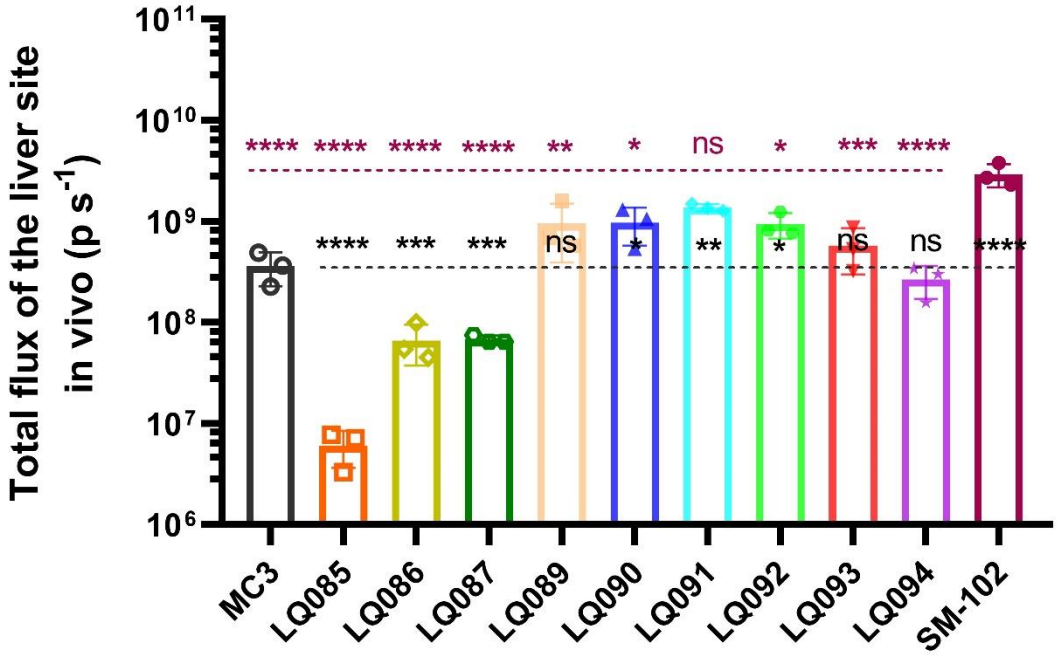
Supplementary Figure 11. Characterization of LNPs for the in vivo distribution test and the acute toxicity test. All data are presented as the mean ± SD (n = 3). Source data are provided as a Source Data file. MC3, DLin-MC3-DMA; PDI, polydispersity index; EE, encapsulation efficiency; LNP, lipid nanoparticle; SD, standard deviation.



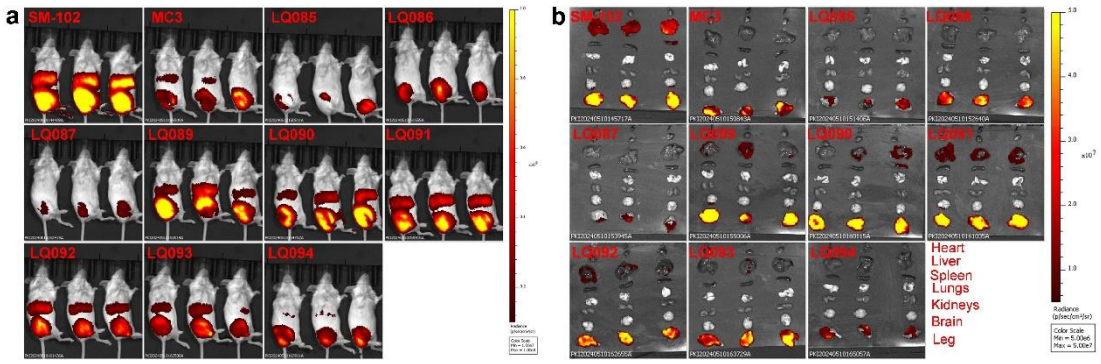
Supplementary Figure 12. Luminescence images of whole body and isolated organs after intravenous administration of luciferase mRNA loaded in different LNPs. (a), Whole-body luminescence. (b), Luminescence from isolated organs. MC3, DLin-MC3-DMA; LNP, lipid nanoparticle.



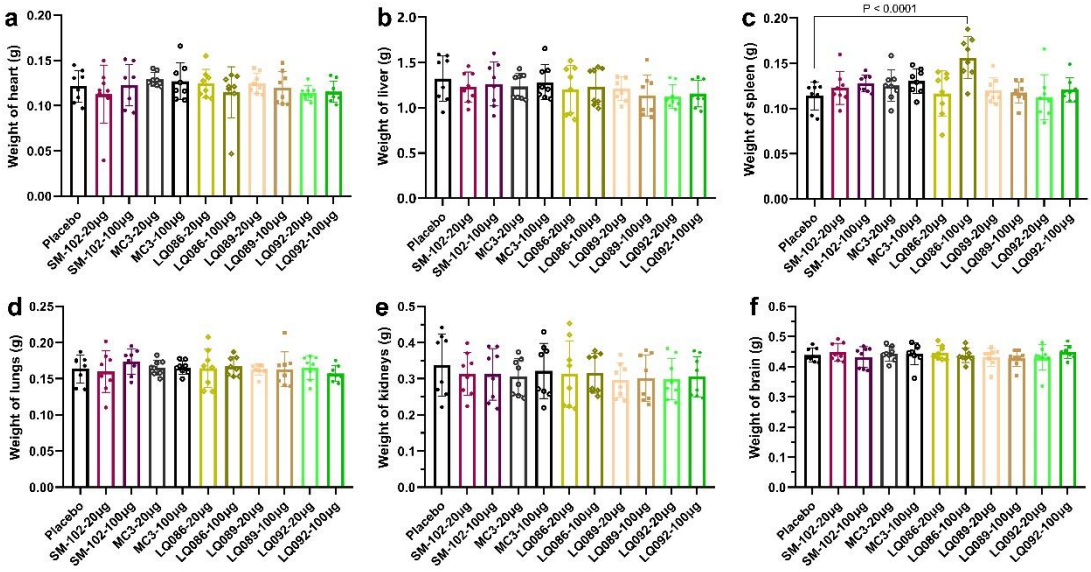
Supplementary Figure 13. Whole-body and organ-specific mRNA expression in mice after intravenous administration. (a), Total flux of luminescence images, each group had three female BALB/c mice and three male ones. (b) The ratio of organ-distributed Cy5-mRNA to administrated Cy5-mRNA after intravenous administration. $\text{Ratio}\ (\%) = \frac{\text{c}\ (Cy5\ mRNA) \cdot V(Homogenate)}{5\ \mu g} \times 100\%$. All data are presented as the mean ± SD (n = 3). Statistical significance was analyzed by one-way ANOVA. (ns, not significant; *, $p < 0.0332$; **, $p < 0.0021$; ***, $p < 0.0002$; ****, $p < 0.0001$. The P makers in black are results of the comparisons with MC3, and those in red are with SM-102. Source data are provided as a Source Data file.). MC3, DLin-MC3-DMA. SD, standard deviation.



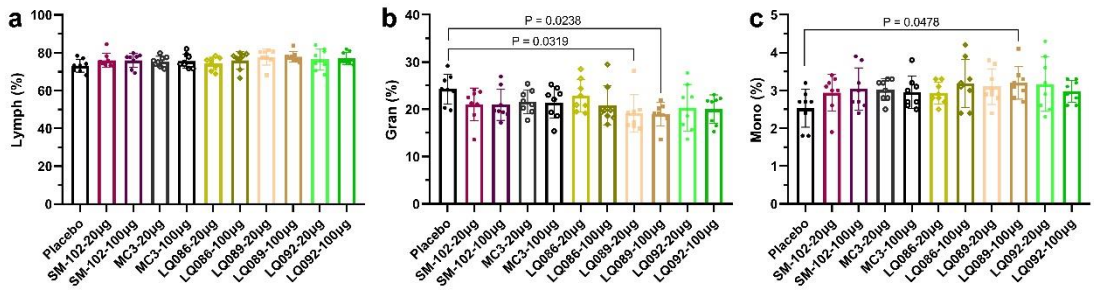
Supplementary Figure 14. Luminescence of the liver site detected in vivo after intramuscular administration of luciferase mRNA loaded in different LNPs. All data are presented as the mean ± SD (n = 3). Statistical significance was analyzed by one-way ANOVA. (ns, not significant; *, $p < 0.0332$; **, $p < 0.0021$; ***, $p < 0.0002$; ****, $p < 0.0001$. The P makers in black are results of the comparisons with MC3, and those in red are with SM-102. Source data are provided as a Source Data file.). MC3, DLin-MC3-DMA; LNP, lipid nanoparticle. SD, standard deviation.



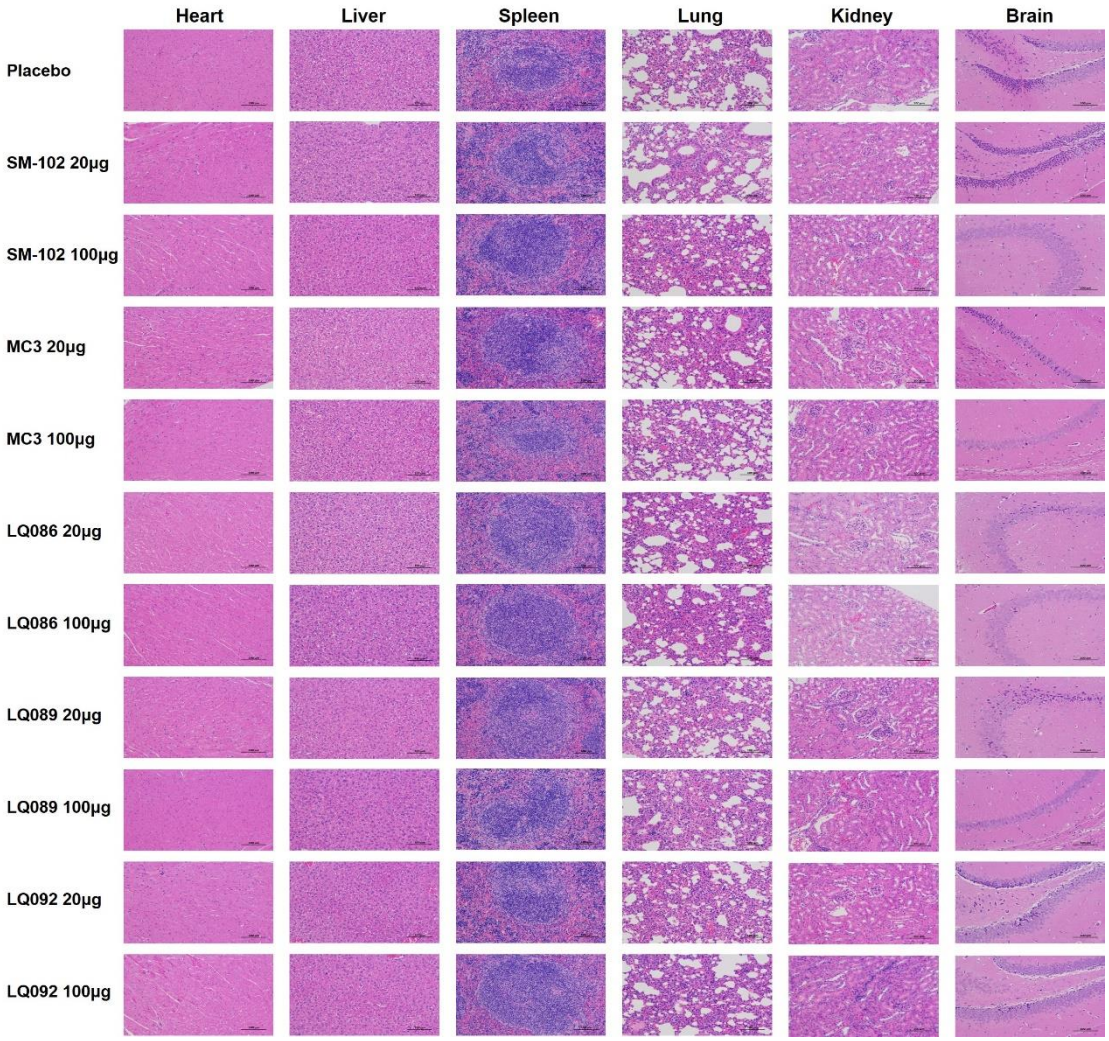
Supplementary Figure 15. Luminescence images of whole body and isolated organs after intramuscular administration of luciferase mRNA loaded in different LNPs. (a), Whole-body luminescence. (b), Luminescence from isolated organs. MC3, DLin-MC3-DMA; LNP, lipid nanoparticle.



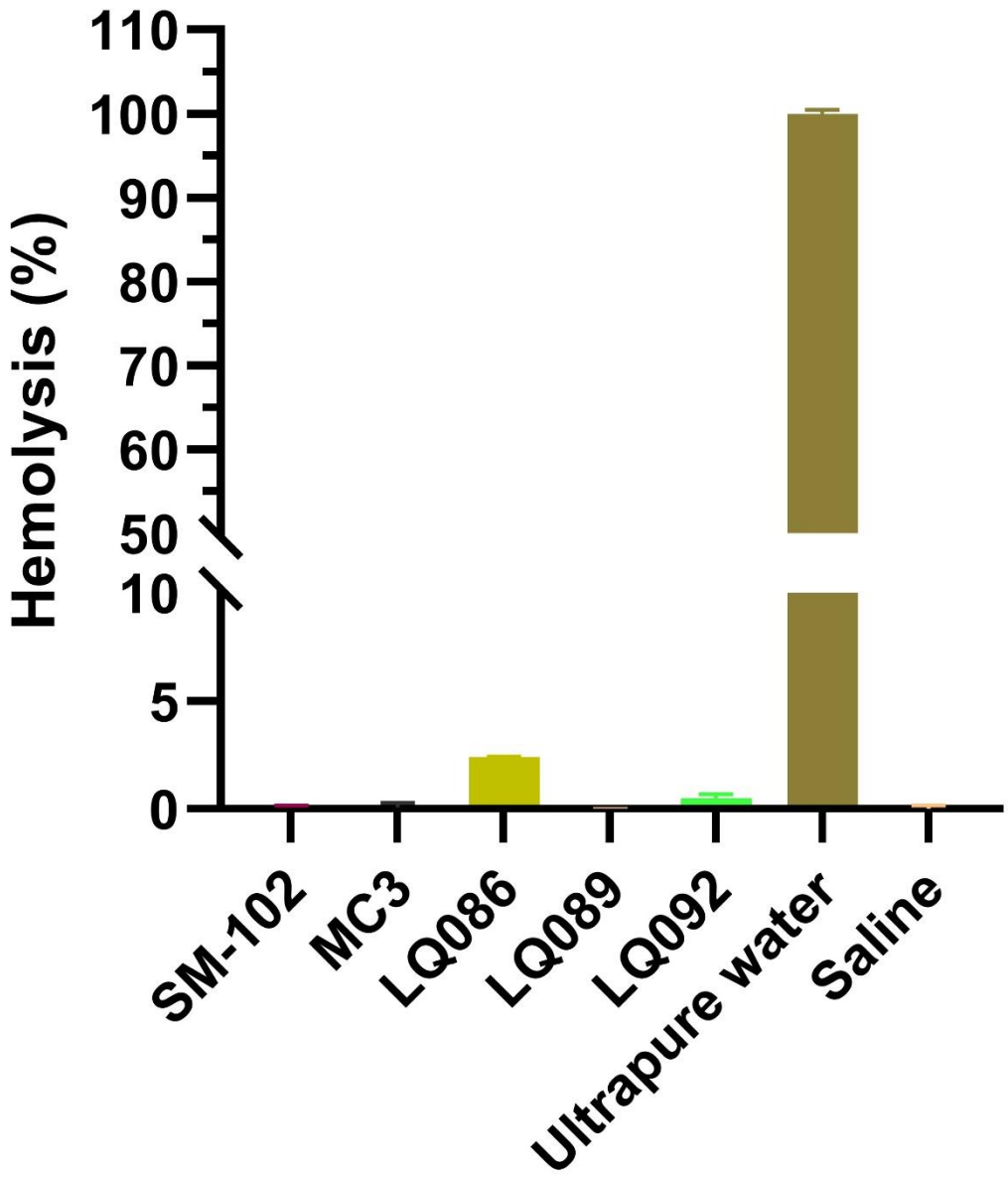
Supplementary Figure 16. Weight of the organs isolated from mice at Day 14 in the acute toxicity test. (a-f), Heart, liver, spleen, lungs, kidneys and brain, respectively. All data are presented as the mean ± SD (n = 8 for each dose, including four females and four males). Statistical significance was analyzed by one-way ANOVA. (unmarked, not significant. Source data are provided as a Source Data file.). MC3, DLin-MC3-DMA. SD, standard deviation.



Supplementary Figure 17. The proportion of different white blood cells. (a), Lymphocyte (%). (b), Granulocyte (%). (c), Monocyte (%). All data are presented as the mean ± SD (n = 8 for each dose, including four females and four males). Statistical significance was analyzed by one-way ANOVA. (unmarked, not significant. Source data are provided as a Source Data file.). MC3, DLin-MC3-DMA. SD, standard deviation.



Supplementary Figure 18. The images of H&E-stained histopathological tissues obtained from the female mices of each group. Scale bar = 100 μm.There were no obvious pathological features such as bleeding, degeneration, necrosis and inflammatory infiltration in the vital organs. MC3, DLin-MC3-DMA.



Supplementary Figure 19. Hemolysis test. Rabbit red blood cells (2 %) and LNPs with mRNA concentration of 50 μg mL^{-1} (100 μL each) were added to a 96-well plate, incubated at 37℃ for 3 h, and then the absorbance of the supernatant at 540 nm was detected. $Hemolysis\ (\%) = \frac{\text{OD}\ (Sample) - \text{Average OD}\ (Saline)}{\text{Average OD}\ (UP\ water)} \times 100\%$. All data are presented as the mean ± SD (n = 3). Source data are provided as a Source Data file. MC3, DLin-MC3-DMA; LNP, lipid nanoparticle. SD, standard deviation.

Supplementary Lipid Synthesis Methods and Characterization

1 Characterization of products

1.1 Thin-Layer Chromatography (TLC)

TLC was used to monitor the reaction and to guide the collection of products during column chromatography. The TLC plates were stained with iodine vapor to facilitate visualization. The intermediate and final products were then collected and stored at -20 °C for further use.

1.2 Nuclear Magnetic Resonance (NMR)

^{1}H NMR and ^{13}C NMR spectra were detected on a Bruker Ascend 600 (600 MHz) NMR spectrometer. Each final product was dissolved in $CDCl_3$ and detected at 25 °C for both ^{1}H and ^{13}C NMR spectra. Chemical shifts (δ) were reported in ppm. NMR spectra were then analyzed by MestReNova 14.

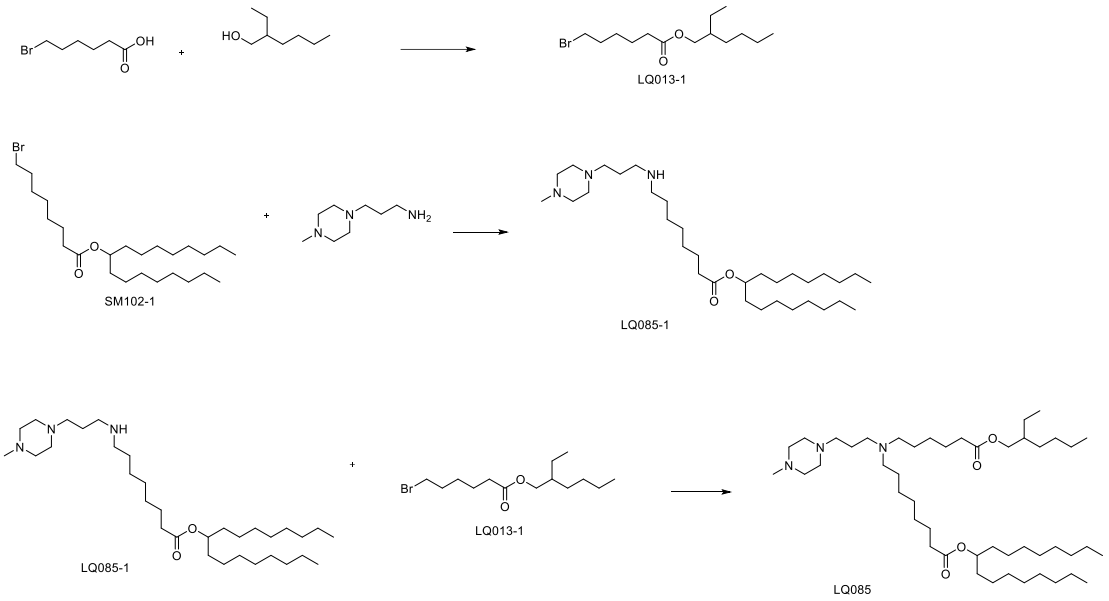
1.3 Liquid Chromatograph-Mass Spectrometry (LC-MS)

Mass spectra of the final lipid molecules were determined by LC-MS employing 1290 Infinity II system (Agilent) and 6545 LC/Q-TOF (Agilent) mass spectrometer equipped with an electrospray ionization (ESI). The LC system consisted of two solvents: Sovent A – deionized water with 15 mM dibutylamine and 25 mM hexafluoroisopropanol, and Sovent B - methanol with 15 mM dibutylamine and 25 mM hexafluoroisopropanol. Each final product was dissolved in methanol and analyzed on a Poroshell 120 EC-C18 column (2.7 μm, 3.0 × 50 mm) with a linear gradient from 100% B - 80% B at a flow rate of 0.4 mL/min for 20 min. The UV absorption at 260 nm was monitored for each injection. Mass spectrum signals were acquired in positive ionization mode, with the dry gas set at 325 °C and 10 L/min, the nebulizer gas maintained at 20 psi, the sheath gas set at 400 °C and 12 L/min, the capillary voltage set at 4000 V, and the MS1 scanning range set between 100 and 3000 m/z.

2 Information of lipids synthesis

2.1 heptadecan-9-yl 8-((6-((2-ethylhexyl)oxy)-6-oxohexyl)(3-(4-methylpiperazin-1-yl)propyl)amino)octanoate (**LQ085**)

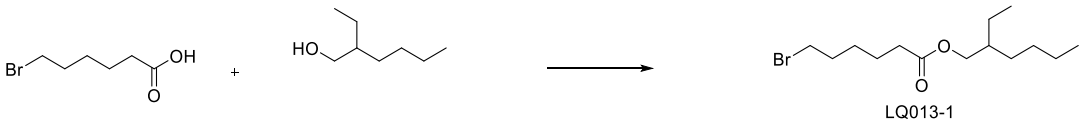
2.1.1 Synthetic route:



2.1.2 Experimental process:

Step 1 Preparation of LQ013-1

Reactive formula:



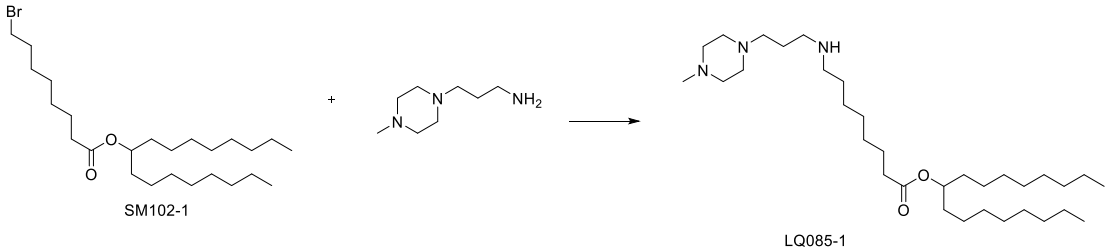
Operation process:

To a solution of 6-bromohexanoic acid (2.34 g, 12 mmol), Dicyclohexylcarbodiimide (DCC, 4.12 g, 20 mmol), and 4-Dimethylaminopyridine (DMAP, 122 mg, 1.2 mmol) in DCM (50 mL) was added 2-ethylhexanol (1.3 g, 10 mmol). The resulting mixture was stirred at room temperature overnight. The crystals were filtered and washed on the filter with DCM (25 mL). The solvent was then removed in vacuo, the residue was

purified by silica gel chromatography (hexanes/EtOAc 20:1, Rf = 0.6) afforded LQ013-1 (2 g) in 66% yield as oil.

Step 2 Preparation of LQ085-1

Reactive formula:

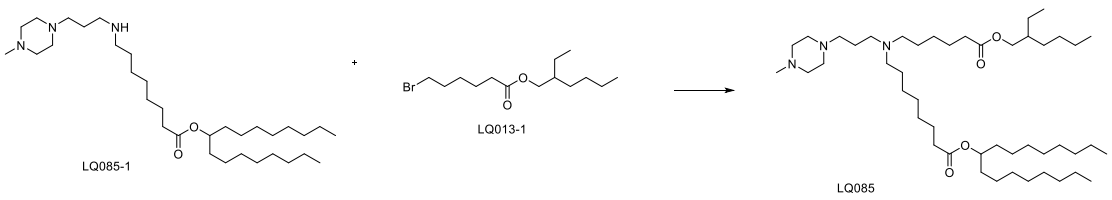


Operation process:

A mixture of SM102-1 (4.62 g, 10 mmol), 1-(3-aminopropyl)-4-methylpiperazine (1.89 g, 12 mmol) and potassium carbonate (2.76 g, 20 mmol) in acetonitrile (40 mL) was stirred at 30 ℃ overnight. The crystals were filtered and washed on the filter with ethyl acetate (25 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 10:1, Rf = 0.4) afforded LQ085-1 (2.5 g) in 46% yield as oil.

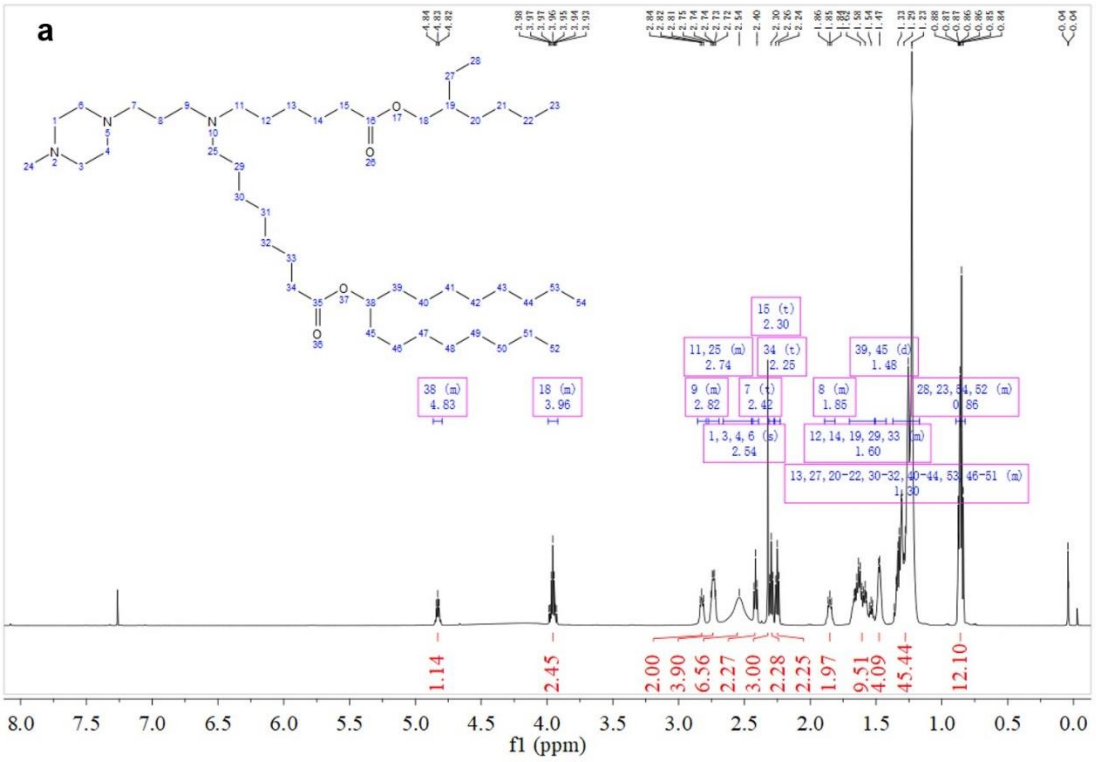
Step 3 Preparation of LQ085

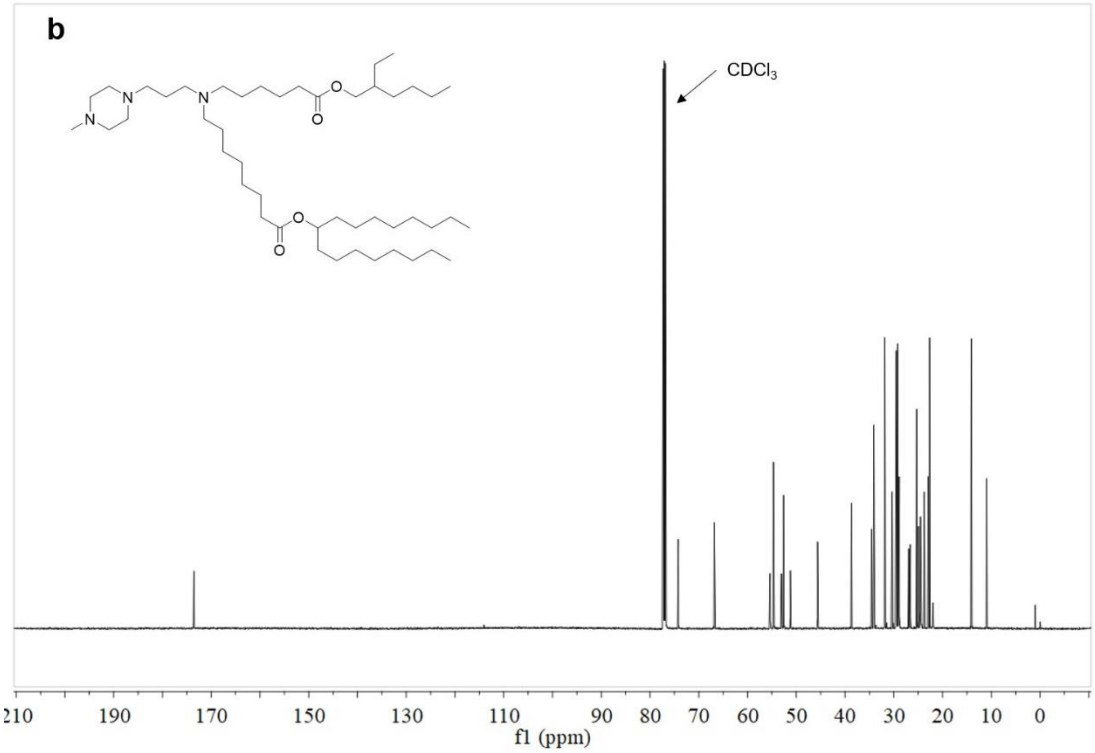
Reactive formula:

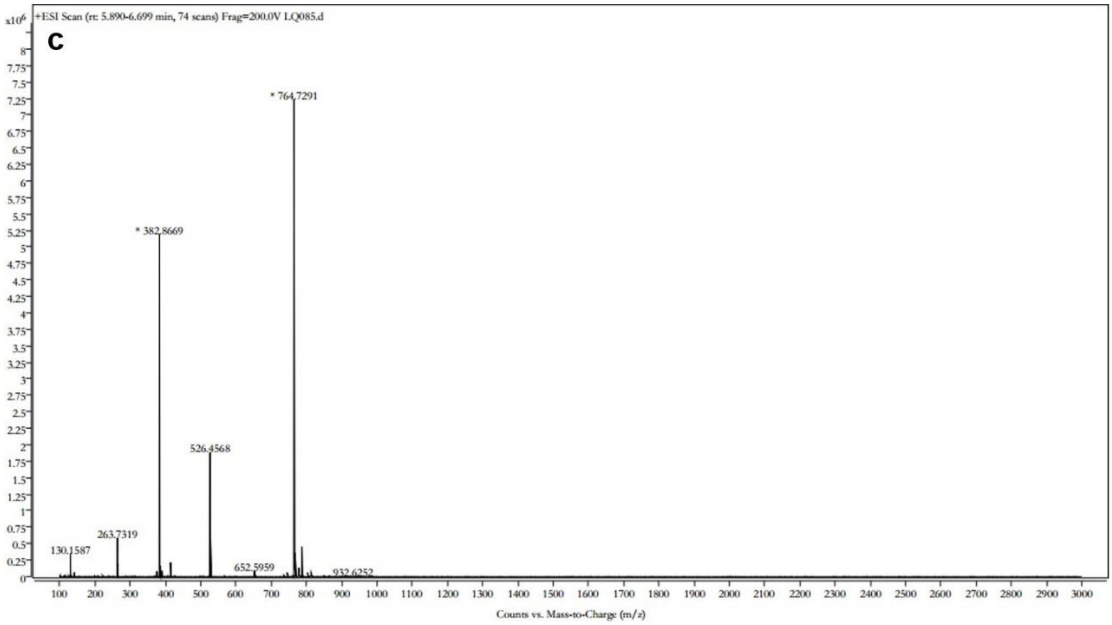


Operation process:

A mixture of LQ085-1 (2.5 g, 4.65 mmol), LQ013-1 (1.7g, 5.58 mmol), potassium carbonate (1.28 g, 9.3 mmol) and potassium iodide (770 mg, 4.45 mmol) in acetonitrile (40 mL) was stirred at 80 ℃ overnight. The crystals were filtered and washed on the filter with ethyl acetate (25 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 10:1, Rf = 0.6) afforded LQ085 (1.5 g) in 42% yield as oil.


a
38 (m)
4.83
18 (m)
3.96
15 (t)
2.30
11, 25 (m)
2.74
34 (t)
2.25
39, 45 (d)
1.48
9 (m)
2.82
7 (t)
2.42
8 (m)
1.85
28, 23, 54, 52 (m)
0.86
1, 3, 4, 6 (m)
2.54
12, 14, 19, 29, 33 (m)
1.60
13, 27, 20-22, 30-32, 40-44, 53, 46-51 (m)
1.30
1.14
2.45
2.00
3.90
6.56
2.27
3.00
2.28
2.25
1.97
9.51
4.09
45.44
12.10
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

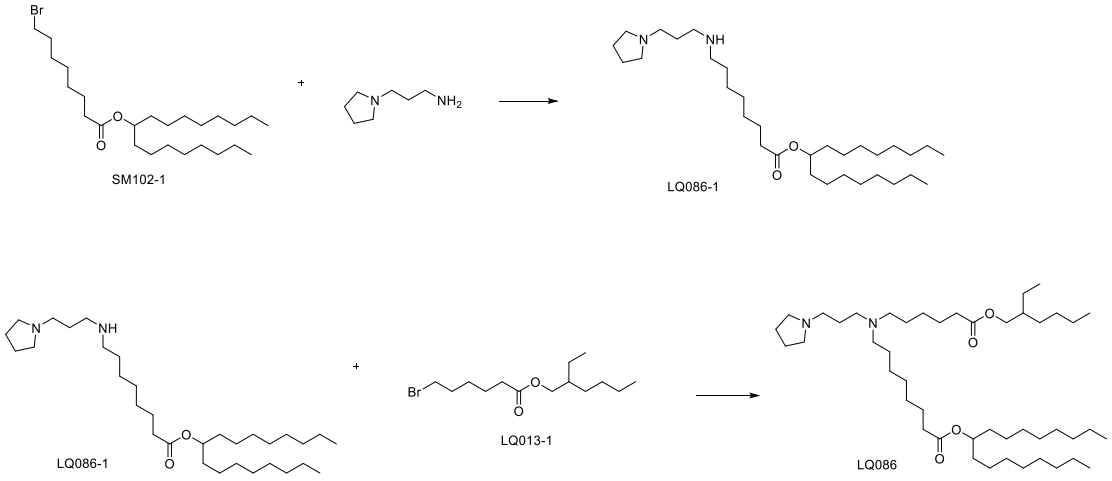

b
CDCl3
210 190 170 150 130 110 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



Supplementary Figure 20. Chemical characterization of LQ085. (a) **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.86 – 4.80 (m, 1H), 3.99 – 3.92 (m, 2H), 2.85 – 2.79 (m, 2H), 2.77 – 2.69 (m, 4H), 2.54 (s, 7H), 2.42 (t, *J* = 6.9 Hz, 2H), 2.30 (t, *J* = 7.4 Hz, 2H), 2.25 (t, *J* = 7.5 Hz, 3H), 1.89 – 1.81 (m, 2H), 1.70 – 1.51 (m, 9H), 1.48 (d, *J* = 5.0 Hz, 4H), 1.37 – 1.17 (m, 45H), 0.89 – 0.82 (m, 12H). (b) **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.63, 173.55, 74.20, 66.82, 54.68, 52.61, 45.62, 38.71, 34.55, 34.11, 34.04, 31.85, 30.38, 29.51, 29.49, 29.22, 29.01, 28.96, 28.90, 26.96, 26.63, 25.30, 24.94, 24.53, 23.76, 22.96, 22.65, 14.11, 14.05, 10.98. (c) **MS** (ESI, m/z) $[M+H]^+$ calculated for $C_{47}H_{93}N_3O_4$, 764.72; Found, 764.7291.

2.2 heptadecan-9-yl 8-((6-((2-ethylhexyl)oxy)-6-oxohexyl)(3-(pyrrolidin-1-yl)propyl)amino)octanoate (**LQ086**)

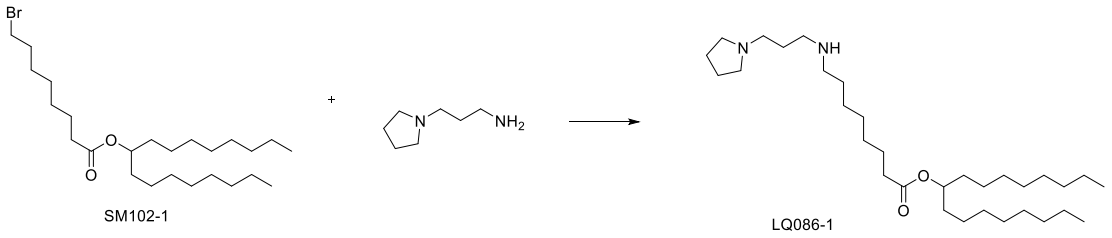
2.2.1 Synthetic route:



2.2.2 Experimental process:

Step 1 Preparation of LQ086-1

Reactive formula:

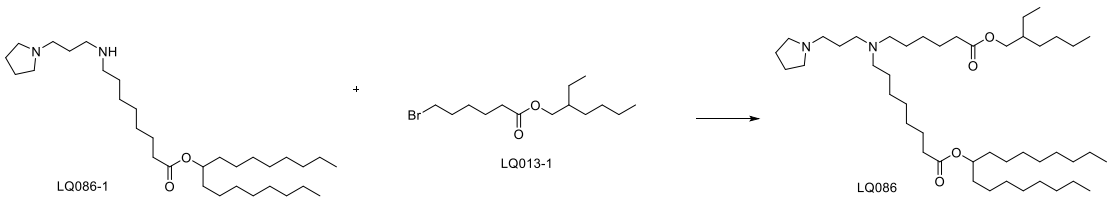


Operation process:

A mixture of SM102-1 (4.15 g, 9 mmol), 1-(3-aminopropyl)pyrrolidine (1.29 g, 10 mmol) and potassium carbonate (2.76 g, 20 mmol) in acetonitrile (40 mL) was stirred at 60 ℃ overnight. The crystals were filtered and washed on the filter with ethyl acetate (25 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 10:1, Rf = 0.15) afforded LQ086-1 (1.7 g) in 37% yield as oil.

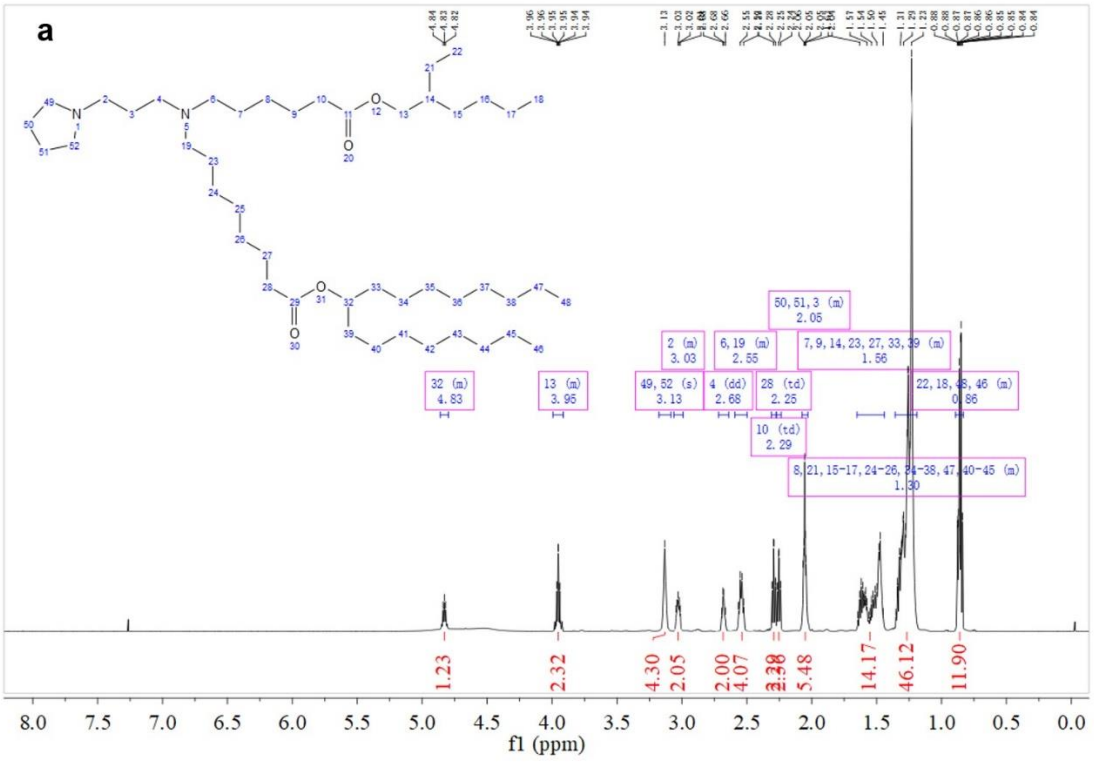
Step 2 Preparation of LQ086

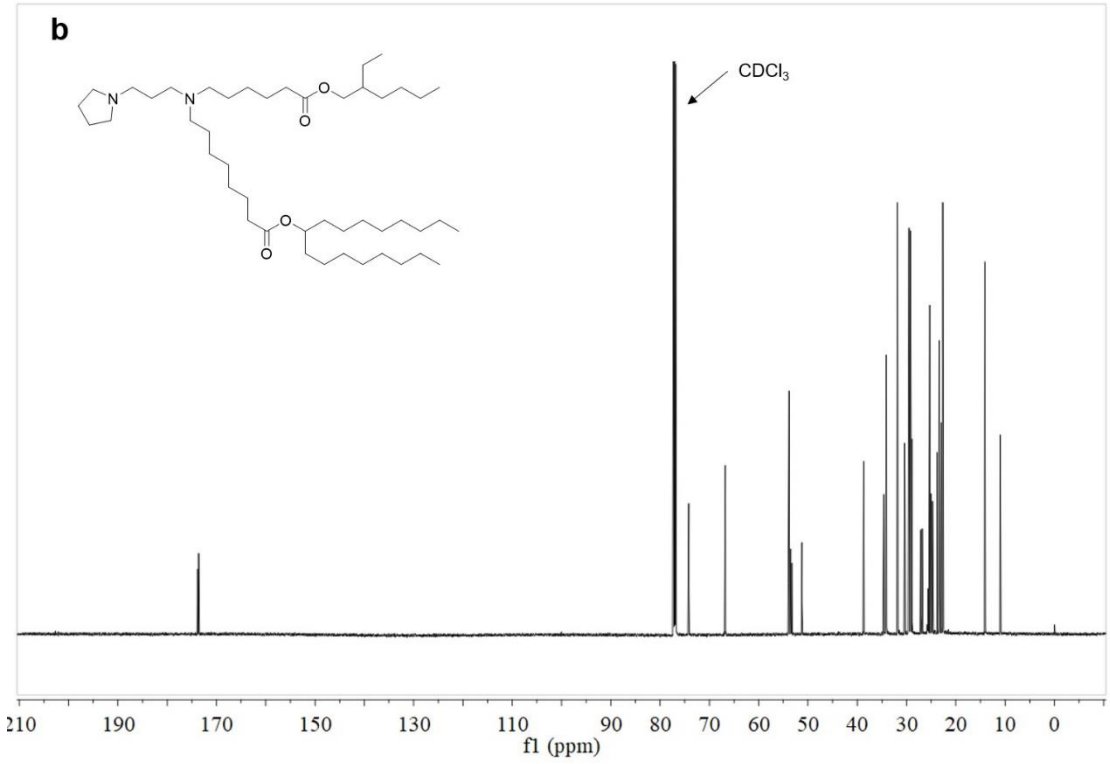
Reactive formula:

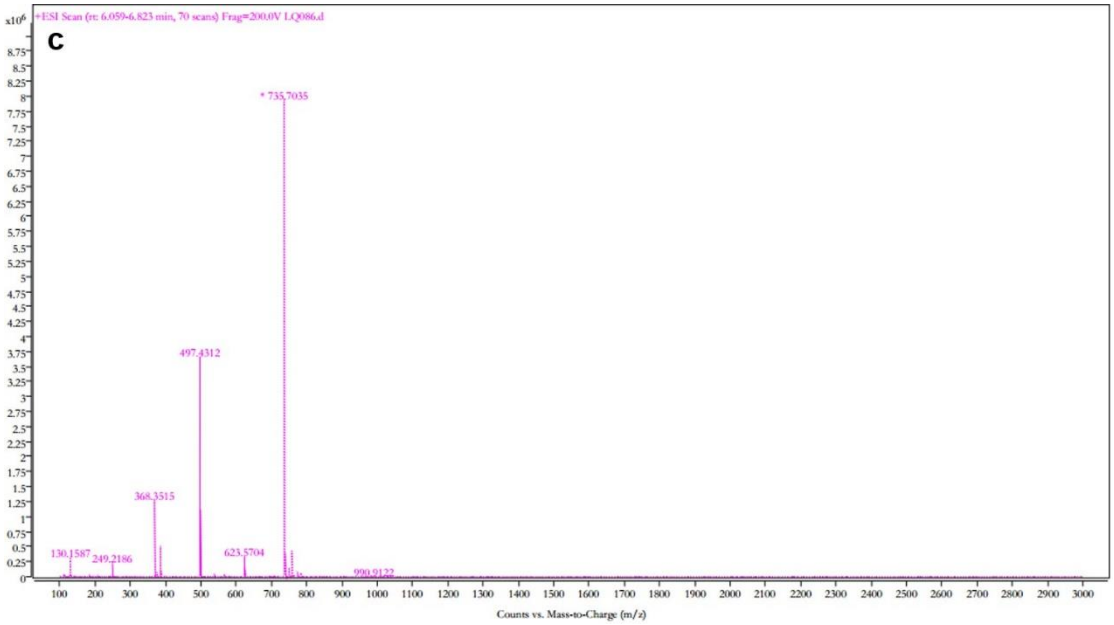


Operation process:

A mixture of LQ086-1 (1.7 g, 3.34 mmol), LQ013-1 (1.23 g, 4 mmol), potassium carbonate (1.8g,13.4 mmol) and potassium iodide (1.1 g, 6.68 mmol) in acetonitrile (80 mL) was stirred at 80 ℃ overnight. The crystals were filtered and washed on the filter with ethyl acetate (30 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/ MeOH 10:1, Rf = 0.6) afforded LQ086 (1.1g) in 45% yield as oil.


a
50, 51, 3 (m)
2.05
2 (m)
3.03
6, 19 (m)
2.55
7, 9, 14, 23, 27, 33, 39 (m)
1.56
32 (m)
4.83
13 (m)
3.95
49, 52 (s)
3.13
4 (dd)
2.68
28 (td)
2.25
22, 18, 46 (m)
0.86
10 (td)
2.29
8, 21, 15-17, 24-26, 34-38, 47, 40-45 (m)
1.30
1.23
2.32
4.30
2.05
2.00
4.07
5.48
14.17
46.12
11.90
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

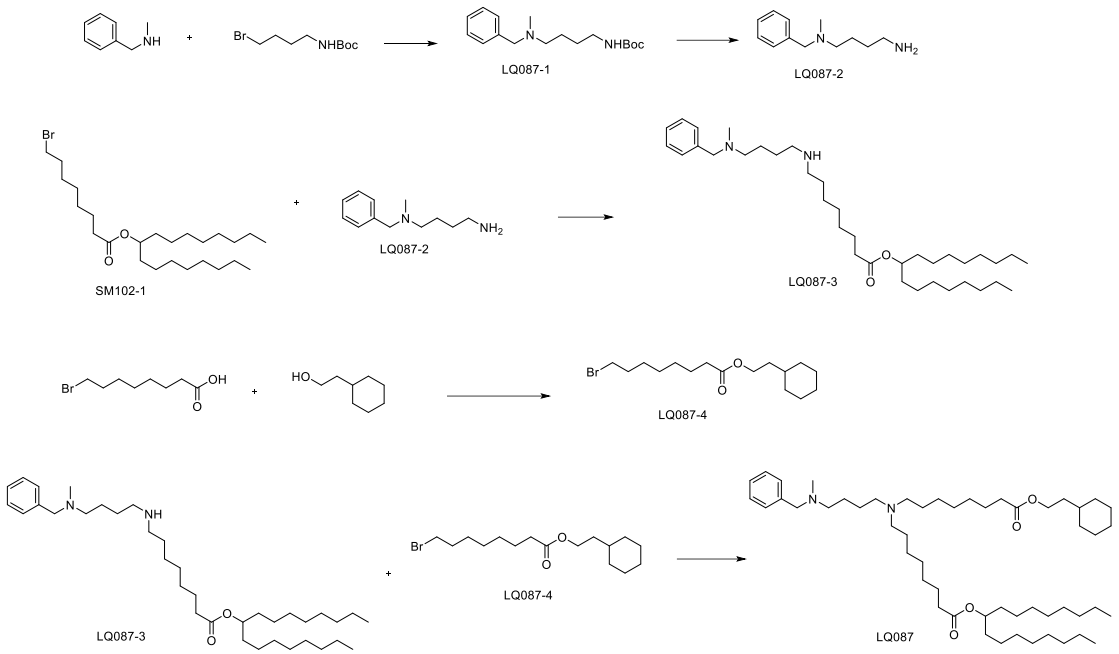

b
CDCl3
210 190 170 150 130 110 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



Supplementary Figure 21. Chemical characterization of LQ086. (a) **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.86 – 4.80 (m, 1H), 3.99 – 3.91 (m, 2H), 3.13 (s, 4H), 3.06 – 2.99 (m, 2H), 2.68 (dd, *J* = 11.1, 6.6 Hz, 2H), 2.59 – 2.50 (m, 4H), 2.29 (td, *J* = 7.4, 0.8 Hz, 2H), 2.25 (td, *J* = 7.7, 1.2 Hz, 2H), 2.07 – 2.03 (m, 5H), 1.65 – 1.44 (m, 14H), 1.36 – 1.19 (m, 46H), 0.89 – 0.83 (m, 12H). (b) **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.80, 173.61, 74.18, 66.76, 53.84, 38.71, 34.61, 34.17, 34.11, 31.84, 30.38, 29.51, 29.49, 29.22, 29.13, 29.10, 28.90, 25.30, 25.00, 24.73, 23.76, 23.40, 22.96, 22.65, 14.10, 14.05, 10.98. (c) **MS** (ESI, m/z) $[M+H]^+$ calculated for $C_{46}H_{90}N_2O_4$, 735.69; Found, 735.7035.

2.3 2-cyclohexylethyl 8-((4-(benzyl(methyl)amino)butyl)(8-(heptadecan-9-yloxy)-8-oxooctyl)amino)octanoate (**LQ087**)

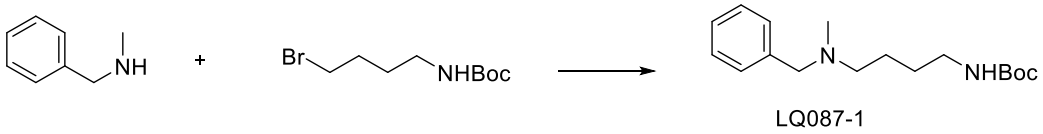
2.3.1 Synthetic route:



2.3.2 Experimental process:

Step 1 Preparation of LQ087-1

Reactive formula:

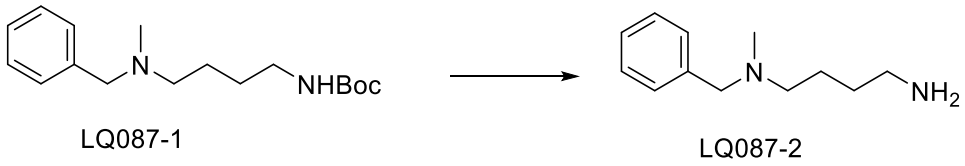


Operation process:

A mixture of N-methylbenzylamine(1.21 g, 10 mmol), tert-butyl N-(4-bromobutyl) carbamic acid (2.52 g, 10 mmol), potassium carbonate (2.76 g, 20 mmol) and potassium iodide (1.66 g, 10 mmol) in acetonitrile (80 mL) was stirred at 60 ℃ overnight. The crystals were filtered and washed on the filter with ethyl acetate (30 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/ MeOH 10:1, Rf = 0.8) afforded LQ087-1 (1.8 g) in 61% yield as oil.

Step 2 Preparation of LQ087-2

Reactive formula:

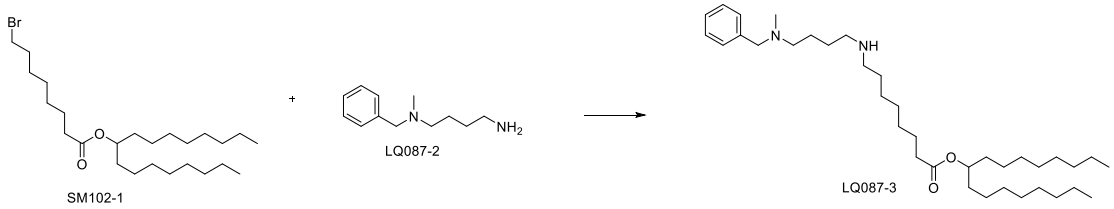


Operation process:

LQ087-1 1.8 g and hydrogen chloride-dioxane solution (4 M) 20 mL were added to the reaction flask and the mixture stirred 2 h at room temperature. The solvent was then removed in vacuo and afforded LQ087-2 (880 mg) in 75% yield as oil.

Step 3 Preparation of LQ087-3

Reactive formula:



Operation process:

A mixture of SM102-1 (1.44 g, 3.12 mmol), LQ087-2 (500 mg, 2.6 mmol) and potassium carbonate (720 mg ,5.2 mmol) in acetonitrile (30 mL) was stirred at 30 ℃ overnight. The crystals were filtered and washed on the filter with ethyl acetate (25 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 10:1, Rf = 0.3) afforded LQ087-3 (760 mg) in 51% yield as oil.

Step 4 Preparation of LQ087-4

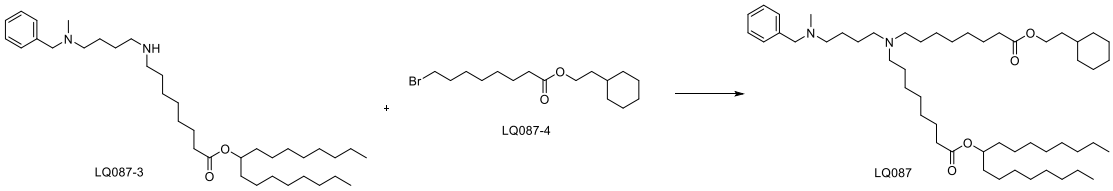
Reactive formula:

Operation process:

To a solution of 8-bromooctanoic acid (2.67 g, 12 mmol), Dicyclohexylcarbodiimide (DCC, 4.12 g, 20 mmol), and 4-Dimethylaminopyridine (DMAP, 122 mg, 1.2 mmol) in DCM (50 mL) was added 2-cyclohexylethyl glycol (1.28 g, 10 mmol). The resulting mixture was stirred at room temperature overnight. The crystals were filtered and washed on the filter with DCM (25 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (hexanes/EtOAc 20:1, Rf = 0.6) afforded LQ087-4 (2.2 g) in 66.7% yield as oil.

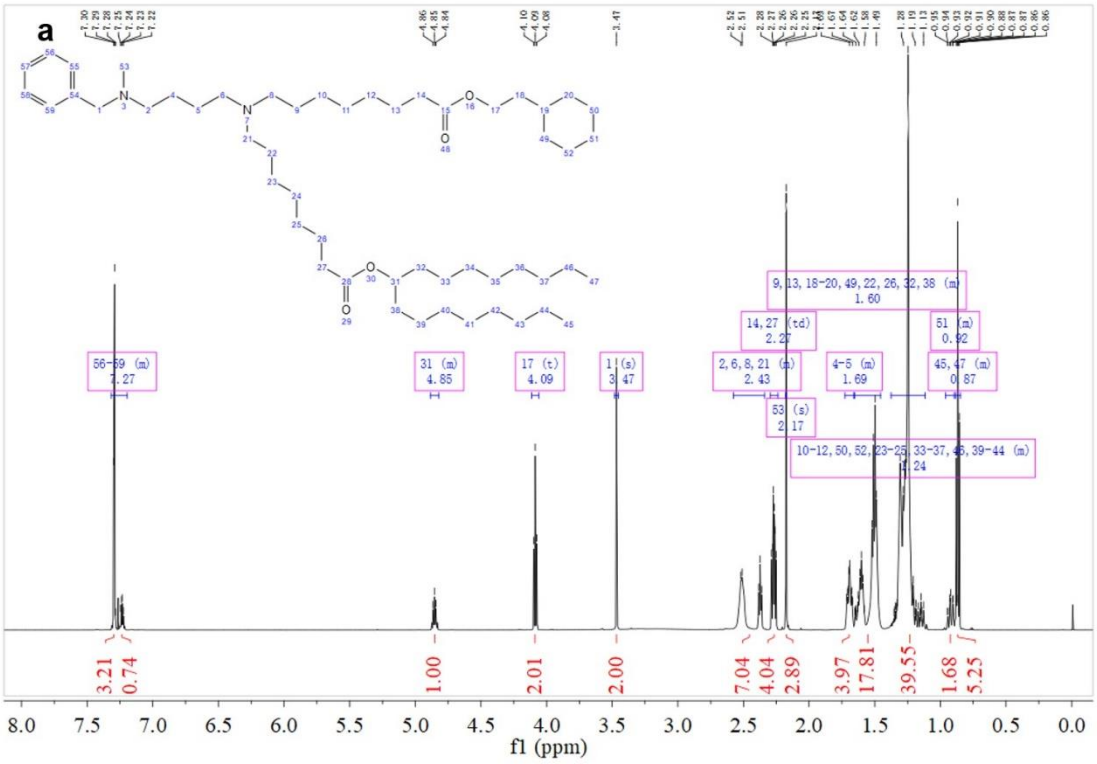
Step 5 Preparation of LQ087

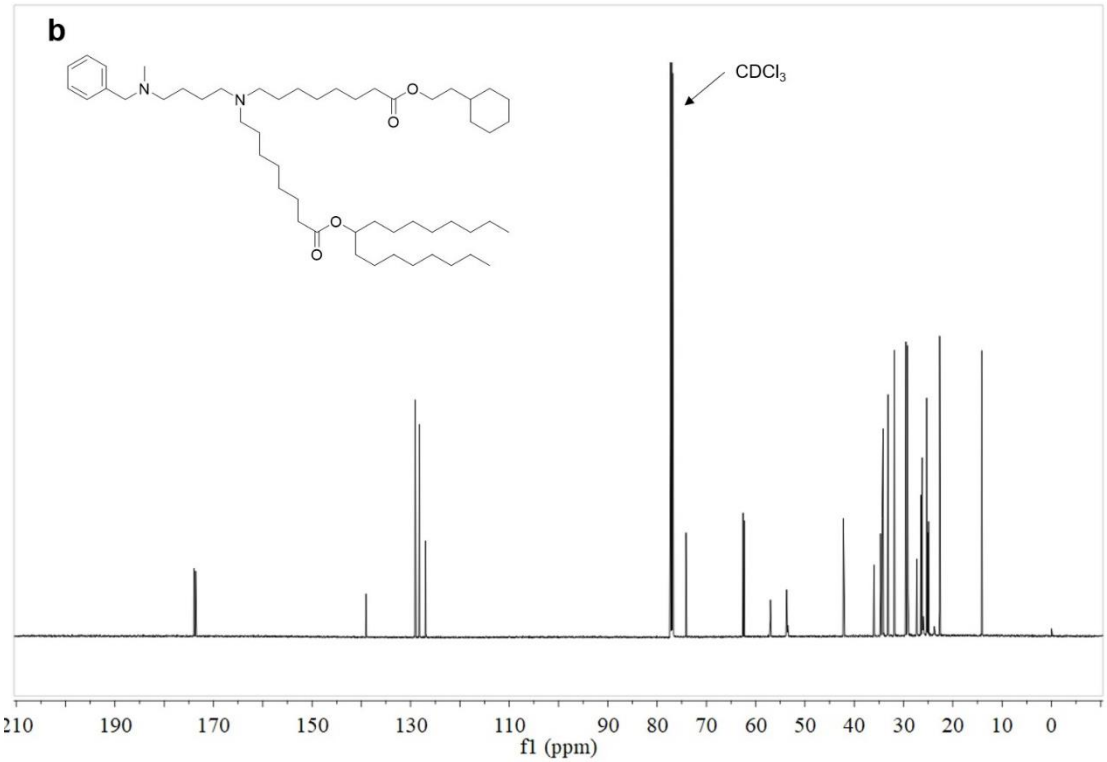
Reactive formula:

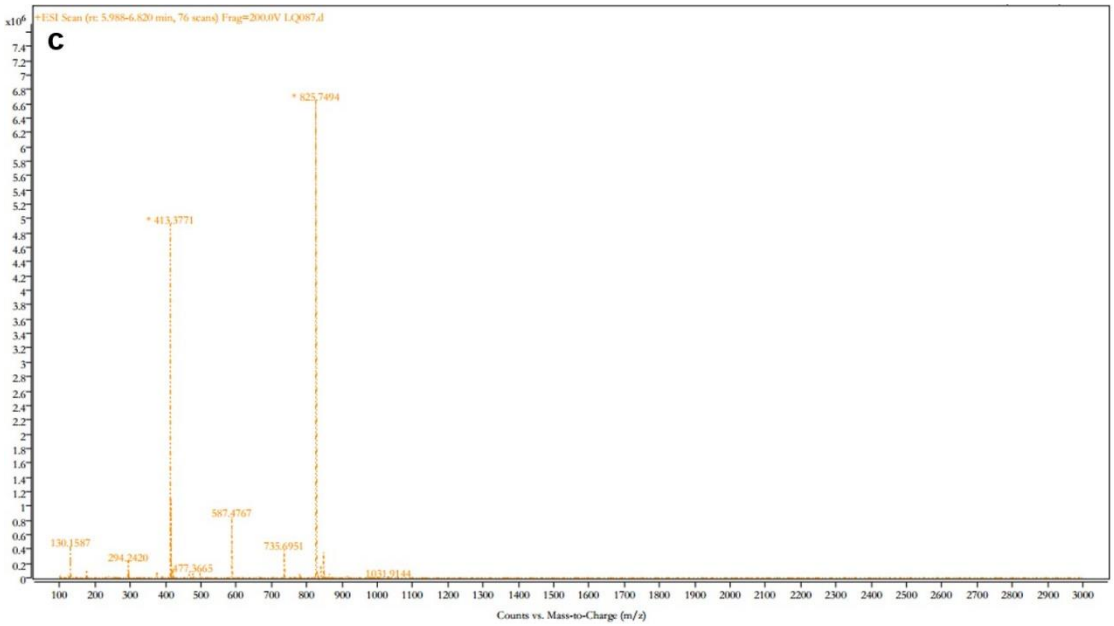


Operation process:

A mixture of LQ087-3(760 mg, 1.32 mmol), LQ087-4(530 mg, 1.6 mmol), potassium carbonate (360 mg, 2.64 mmol) and potassium iodide (220 mg, 1.32 mmol) in acetonitrile (20 mL) was stirred at 60 ℃ overnight. The crystals were filtered and washed on the filter with ethyl acetate (10 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/ MeOH 10:1, Rf = 0.5) afforded LQ087 (420 mg) in 38% yield as oil.


a
56-59 (m)
7.27
31 (m)
4.85
17 (t)
4.09
1 (s)
3.47
9,13,18-20,49,22,26,32,38 (m)
1.60
14,27 (td)
2.27
2,6,8,21 (m)
2.43
4-5 (m)
1.69
51 (m)
0.92
45,47 (m)
0.87
53 (s)
2.17
10-12,50,52,23-25,33-37,46,39-44 (m)
1.24
3.21
0.74
1.00
2.01
2.00
7.04
4.04
2.89
3.97
17.81
39.55
1.68
5.25
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

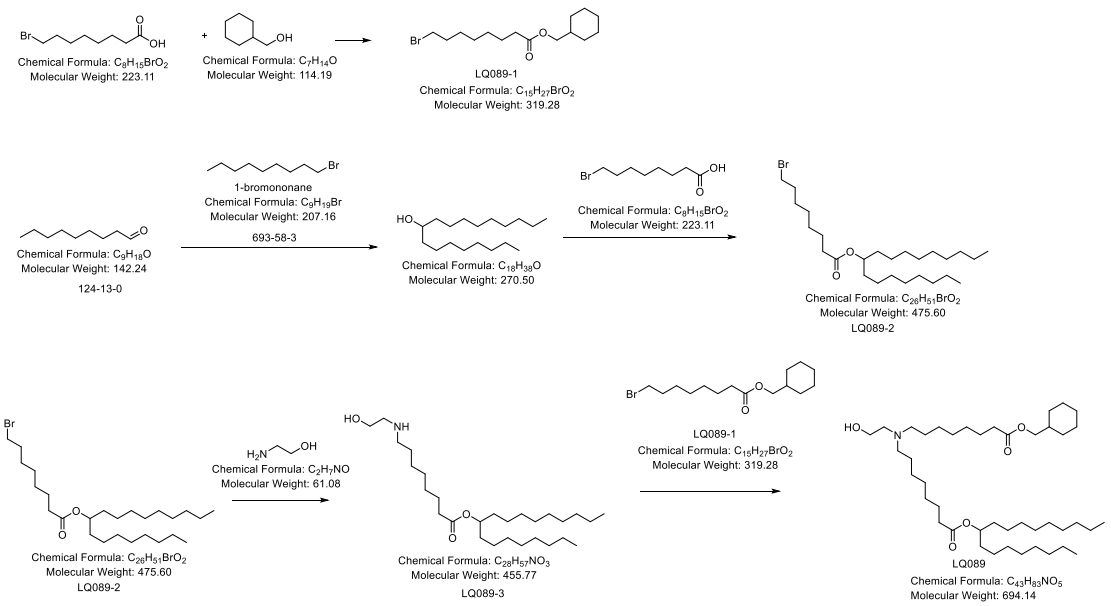

b
CDCl3
210 190 170 150 130 110 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



Supplementary Figure 22. Chemical characterization of LQ087. (a) **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.32 – 7.19 (m, 4H), 4.88 – 4.82 (m, 1H), 4.09 (t, *J* = 6.9 Hz, 2H), 3.47 (s, 2H), 2.58 – 2.34 (m, 7H), 2.27 (td, *J* = 7.5, 5.3 Hz, 4H), 2.17 (s, 3H), 1.73 – 1.66 (m, 4H), 1.65 – 1.46 (m, 18H), 1.38 – 1.12 (m, 40H), 0.96 – 0.89 (m, 2H), 0.89 – 0.85 (m, 5H). (b) **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.94, 173.63, 139.06, 129.07, 128.20, 126.95, 74.14, 62.55, 62.33, 42.21, 36.01, 34.67, 34.58, 34.35, 34.14, 33.16, 31.87, 29.54, 29.51, 29.24, 29.19, 29.16, 29.14, 29.10, 27.30, 26.48, 26.22, 25.32, 25.08, 24.91, 22.67, 14.12. (c) **MS** (ESI, m/z) $[M+H]^+$ calculated for $C_{53}H_{96}N_2O_4$, 825.74; Found, 825.7494.

2.4 cyclohexylmethyl 8-((2-hydroxyethyl)(8-(octadecan-9-yloxy)-8-oxooctyl)amino)octanoate (**LQ089**)

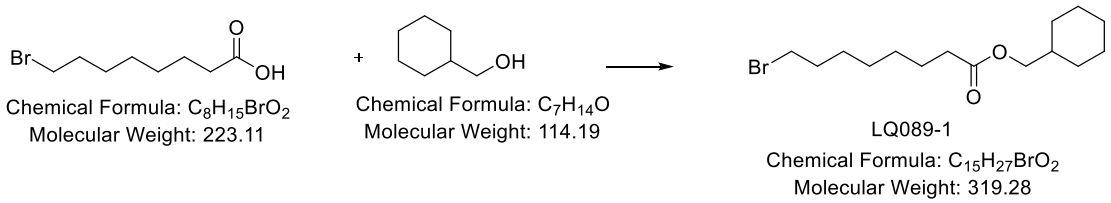
2.4.1 Synthetic route:



2.4.2 Experimental process:

Step 1 Preparation of LQ089-1

Reactive formula:

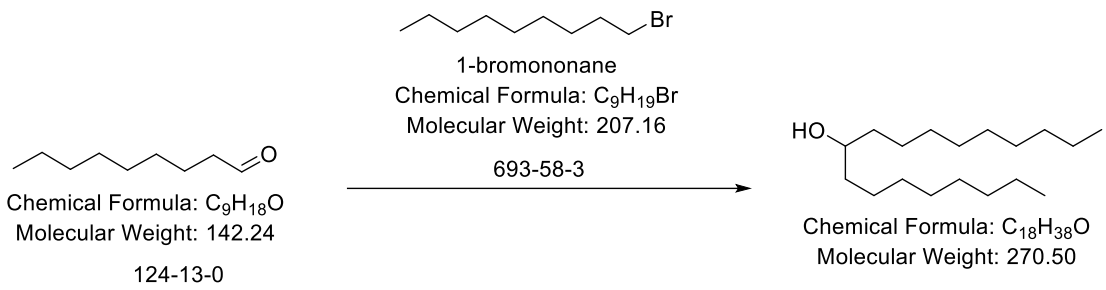


Operational process:

To a solution of 8-bromooctanoic acid (2.2 g, 9.7 mmol), Dicyclohexylcarbodiimide (DCC, 3.6 g, 17.6 mmol), and 4-Dimethylaminopyridine (DMAP, 110 mg, 0.9 mmol) in DCM (50 mL) was added cyclohexanol (1 g, 8.8 mmol). The resulting mixture was stirred at room temperature overnight. The crystals were filtered and washed on the filter with DCM (25 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (hexanes/EtOAc 20:1, Rf = 0.6) afforded LQ089-1 (2.6 g) in 93% yield as oil.

Step 2 Preparation of 9-octadecanol.

Reactive formula:

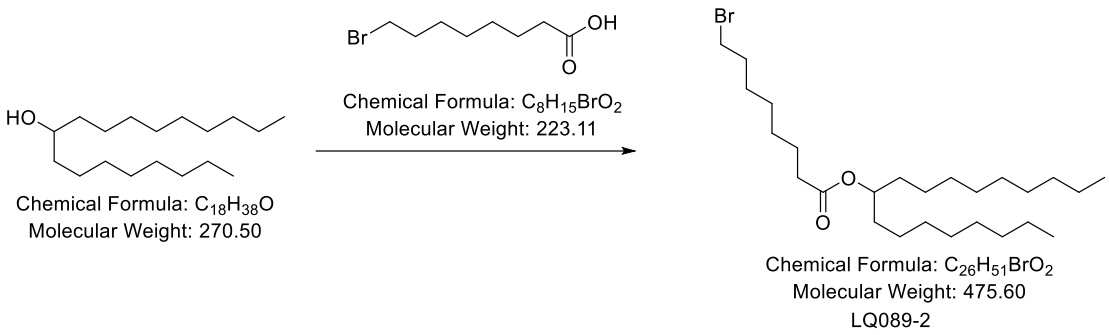


Operational process:

Magnesium turnings and anhydrous tetrahydrofuran were added to the reaction flask under nitrogen protection. The mixture was heated to 45 °C and 1-bromodecane was slowly added dropwise, triggering a Grignard reaction resulting in temperature increase and reflux. After the addition was complete, the mixture was kept at 45 °C for 2 hours, then cooled to 2-10 °C and decanal was slowly added dropwise. After the addition was complete, the mixture was stirred at room temperature for 12 hours, The reaction mixture was cooled to 2-10 °C, and 100 mL of 1 N hydrochloric acid was slowly added dropwise. After stirring for 30 minutes, 600 mL of ethyl acetate and 1 L of water were added for extraction. The organic phase was dried over anhydrous sodium sulfate and then dried by rotary evaporation. The resulting crude product was purified by column chromatography (hexanes/EtOAc 20:1, Rf = 0.3), yielding 81 g of white solid with a yield of 85%.

Step 3 Preparation of LQ089-2.

Reactive formula:

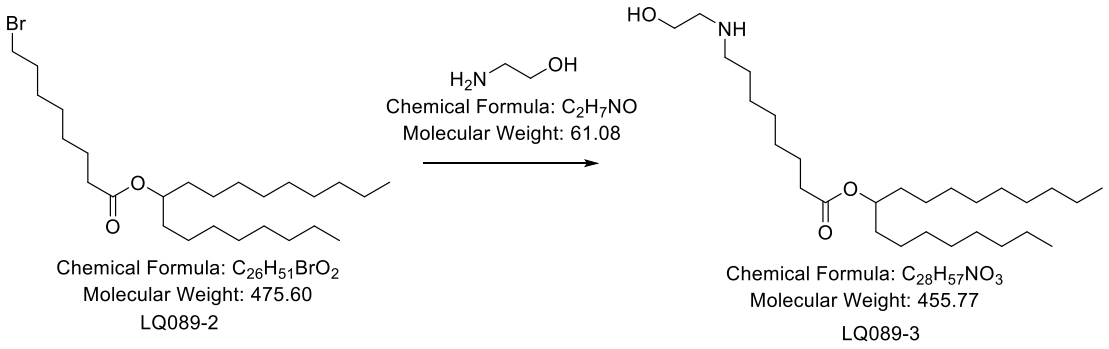


Operational process:

To a solution of 8-bromooctanoic acid (2.45 g, 11 mmol), Dicyclohexylcarbodiimide (DCC, 4.12 g, 20 mmol), and 4-Dimethylaminopyridine (DMAP, 122 mg, 1 mmol) in DCM (50 mL) was added 9-octadecanol (2.7 g, 10 mmol). The resulting mixture was stirred at room temperature overnight. The crystals were filtered and washed on the filter with DCM (25 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (hexanes/EtOAc 20:1, Rf = 0.6) afforded LQ089-2 (3.8 g) in 80% yield as oil.

Step 4 Preparation of LQ089-3

Reactive formula：



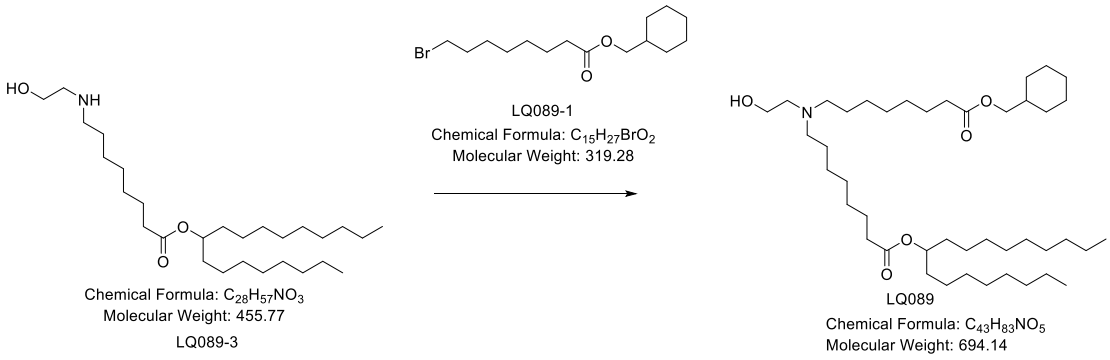
Operation process:

A mixture of LQ089-2 (3.8 g, 8 mmol) and ethanolamine (4.9g, 80 mmol) in acetonitrile (40 mL) was stirred at 30 ℃ overnight. The reaction mixture was diluted with 100 mL

of ethyl acetate and washed twice with 100 mL of water, and the organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 10:1, Rf = 0.4) afforded LQ089-3 (2.9 g) in 80% yield as oil.

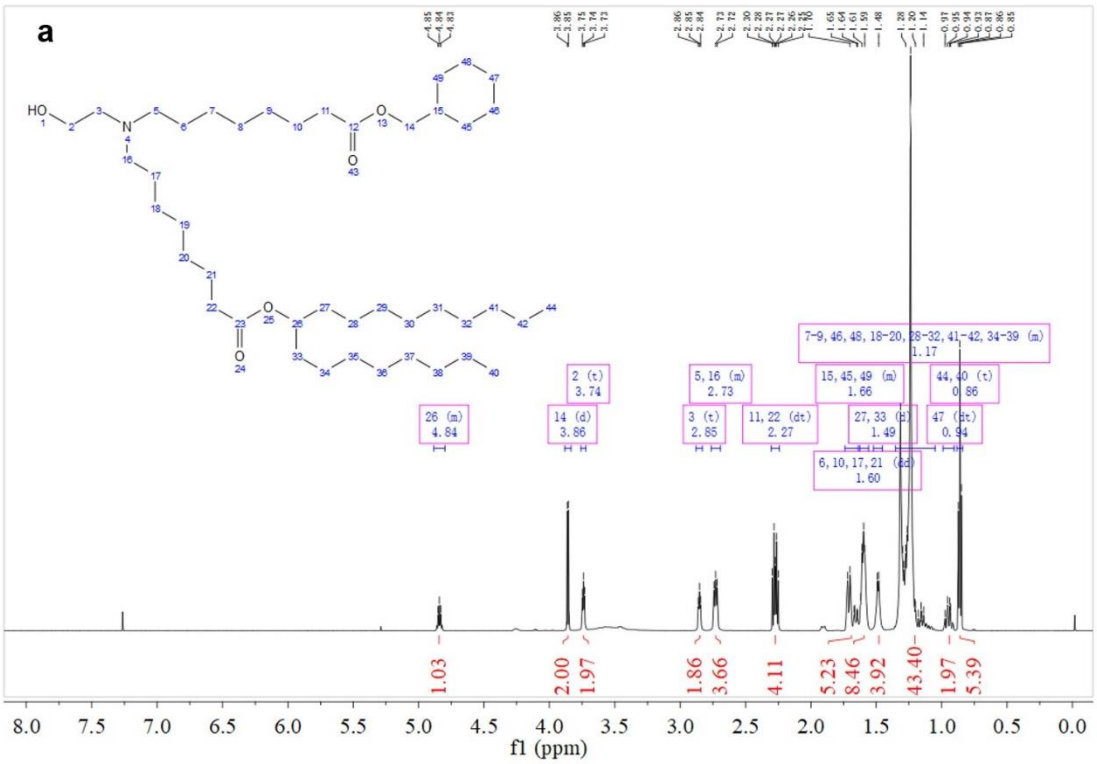
Step 5 Preperation of LQ089

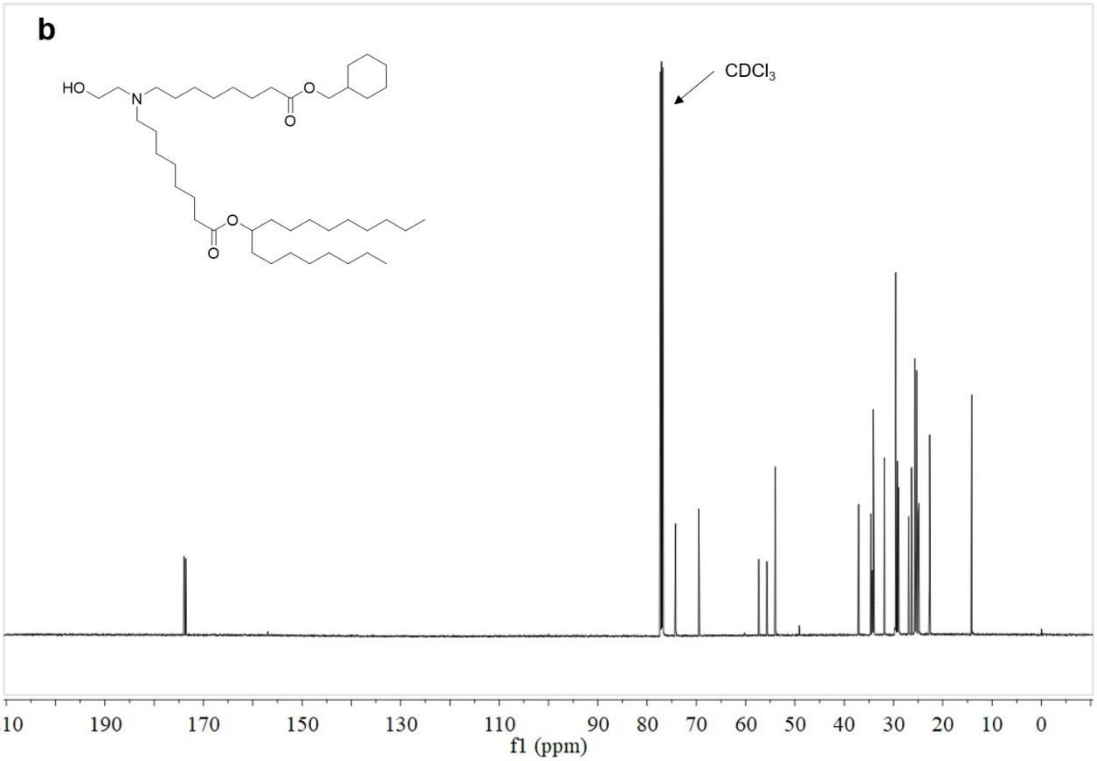
Reactive formula：

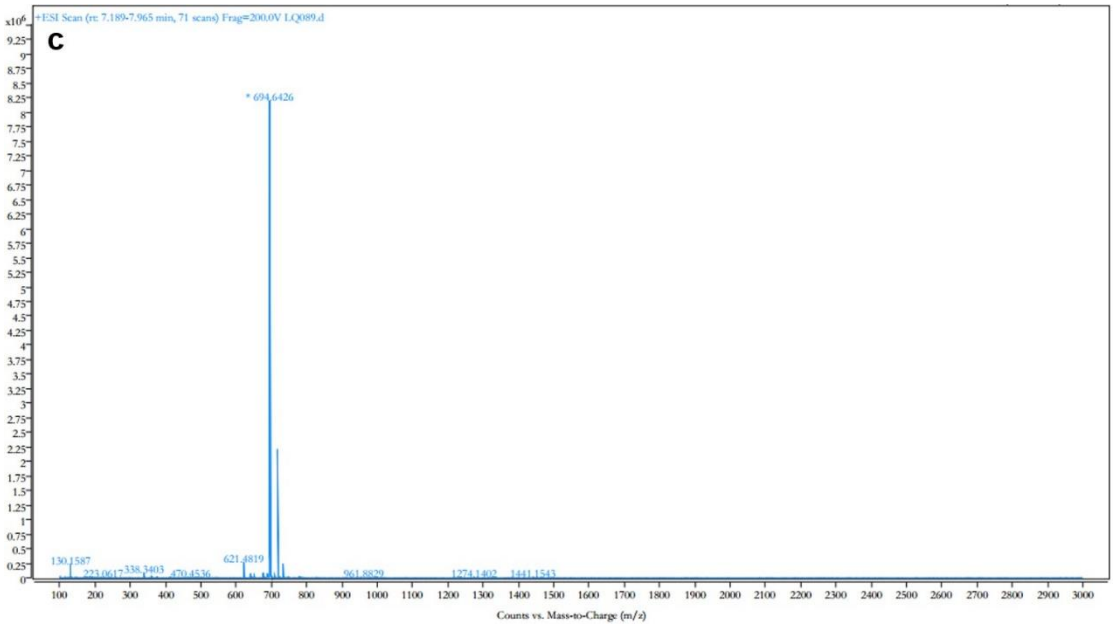


Operation process:

A mixture of LQ089-3 (3.8 g, 8.3 mmol), LQ089-1(3.2 g, 10 mmol), potassium carbonate (2.2 g, 16 mmol), and potassium iodide (1.3 g, 8 mmol) in acetonitrile (40 mL) was stirred at 80 ℃ overnight. The crystals were filtered and washed on the filter with ethyl acetate (20 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 10:1, Rf = 0.6) afforded LQ089 (3 g) in 52% yield as oil.


a
26 (m)
4.84
14 (d)
3.86
2 (t)
3.74
3 (t)
2.85
5,16 (m)
2.73
11,22 (dt)
2.27
6,10,17,21 (dd)
1.60
27,33 (m)
1.49
15,45,49 (m)
1.66
47 (tt)
0.94
44,40 (t)
0.86
7-9,46,48,18-20,28-32,41-42,34-39 (m)
1.17
1.03
2.00
1.97
1.86
3.66
4.11
5.23
8.46
3.92
43.40
1.97
5.39
f1 (ppm)

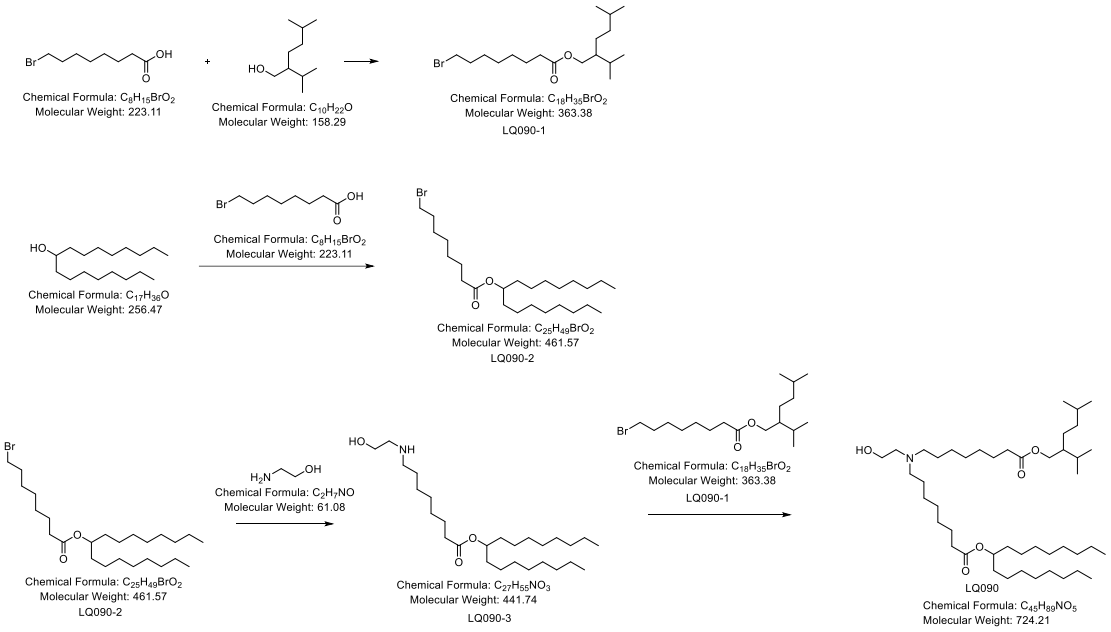

b
CDCl3
f1 (ppm)



Supplementary Figure 23. Chemical characterization of LQ089. (a) **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.88 – 4.80 (m, 1H), 3.86 (d, *J* = 6.6 Hz, 2H), 3.74 (t, 2H), 2.85 (t, *J* = 5.0 Hz, 2H), 2.76 – 2.69 (m, 4H), 2.27 (dt, *J* = 12.2, 7.5 Hz, 4H), 1.74 – 1.64 (m, 5H), 1.60 (dd, *J* = 12.5, 5.8 Hz, 8H), 1.49 (d, *J* = 6.0 Hz, 4H), 1.35 – 1.05 (m, 43H), 0.94 (dt, *J* = 12.2, 7.4 Hz, 2H), 0.86 (t, *J* = 7.0 Hz, 5H). (b) **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.95, 173.61, 74.20, 69.50, 57.37, 53.95, 37.10, 34.60, 34.26, 34.12, 31.89, 31.86, 29.68, 29.54, 29.53, 29.50, 29.31, 29.24, 29.02, 29.00, 28.97, 28.94, 26.95, 26.92, 26.36, 25.67, 25.40, 25.32, 24.98, 24.85, 22.68, 22.66, 14.12. (c) **MS** (ESI, m/z) $[M+H]^+$ calculated for $C_{43}H_{83}NO_5$, 694.63; Found, 694.6426.

2.5 heptadecan-9-yl 8-((2-hydroxyethyl)(8-((2-isopropyl-5-methylhexyl)oxy)-8-oxooctyl)amino)octanoate (**LQ090**)

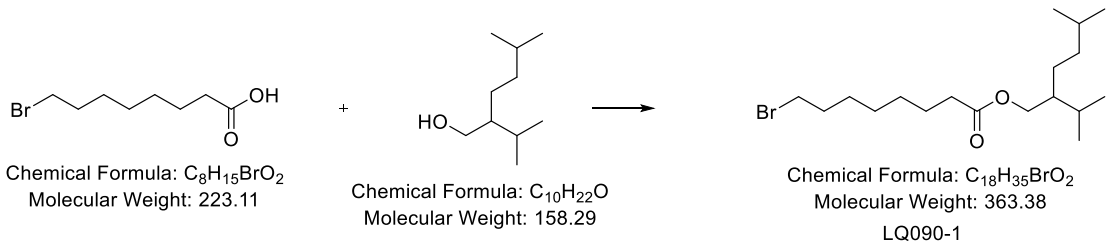
2.5.1 Synthetic route:



2.5.2 Experimental process:

Step 1 Preparation of LQ090-1

Reaction formula:

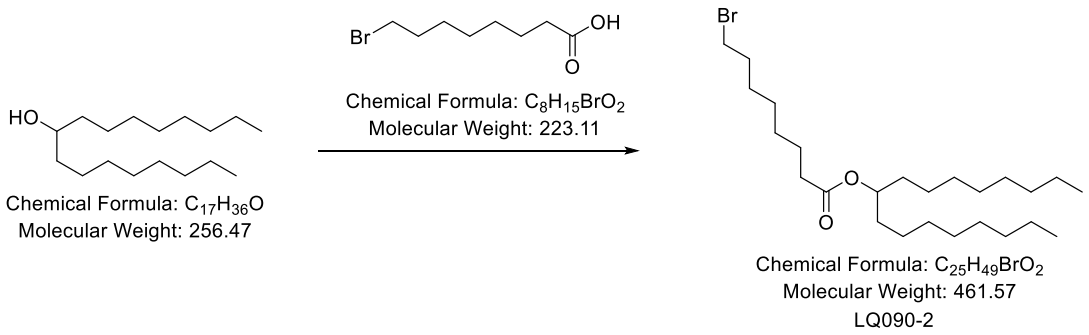


Operation process:

To a solution of 8-bromooctanoic acid (1.56 g, 7 mmol), Dicyclohexylcarbodiimide (DCC, 2.6 g, 12.6 mmol), and 4-Dimethylaminopyridine (DMAP, 80 mg, 0.63 mmol) in DCM (50 mL) was added tetrahydrofurfuryl alcohol (1 g, 6.3 mmol). The resulting mixture was stirred at room temperature overnight. The crystals were filtered and washed on the filter with DCM (25 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (hexanes/EtOAc 20:1, Rf = 0.65) afforded LQ090-1 (2 g) in 87% yield as oil.

Step 2 Preparation of LQ090-2

Reaction formula:

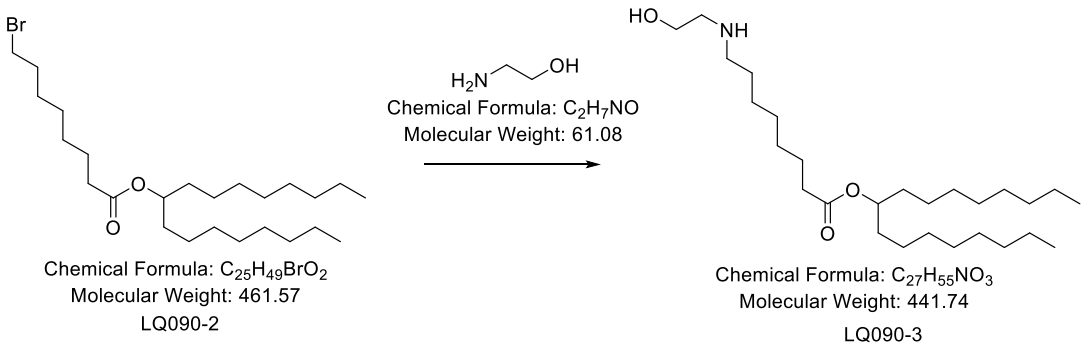


Operation process:

To a solution of 8-bromooctanoic acid (2.45 g, 11 mmol), Dicyclohexylcarbodiimide (DCC, 4.12 g, 20 mmol), and 4-Dimethylaminopyridine (DMAP, 122 mg, 1 mmol) in DCM (50 mL) was added 9-heptadecanol (2.6 g, 10 mmol). The resulting mixture was stirred at room temperature overnight. The crystals were filtered and washed on the filter with DCM (25 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (hexanes/EtOAc 20:1, Rf = 0.6) afforded LQ090-2 (3.6 g) in 78% yield as oil.

Step 3 Preparation of LQ090-3

Reactive formula:

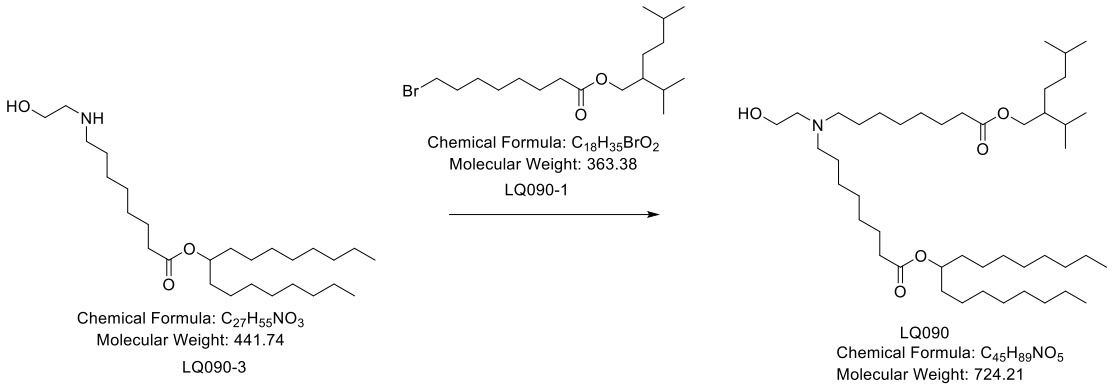


Operation process:

A mixture of LQ090-2 (3.6 g, 8 mmol) and ethanolamine (4.9 g, 80 mmol) in acetonitrile (40 mL) was stirred at 30 ℃ overnight. The reaction mixture was diluted with 100 mL of ethyl acetate, and washed twice with 100 mL of water, and the organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 10:1, Rf=0.4) afforded LQ090-3 (2.8 g) in 79% yield as oil.

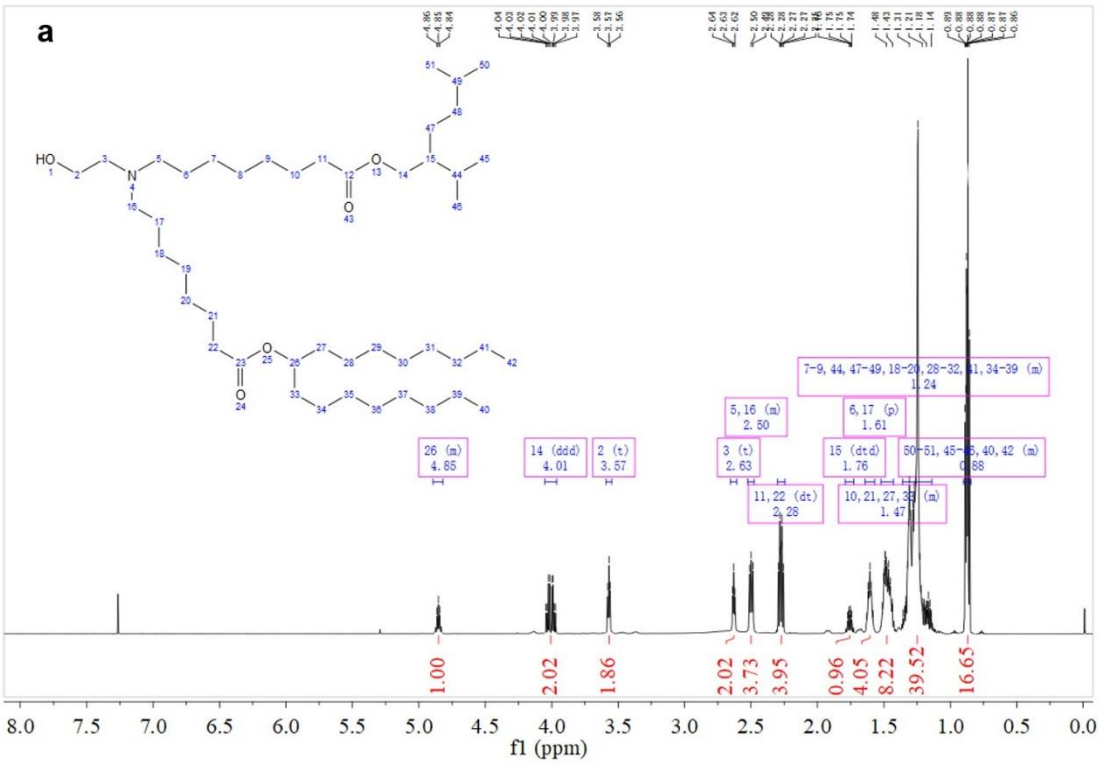
Step 4 Preparation of LQ090

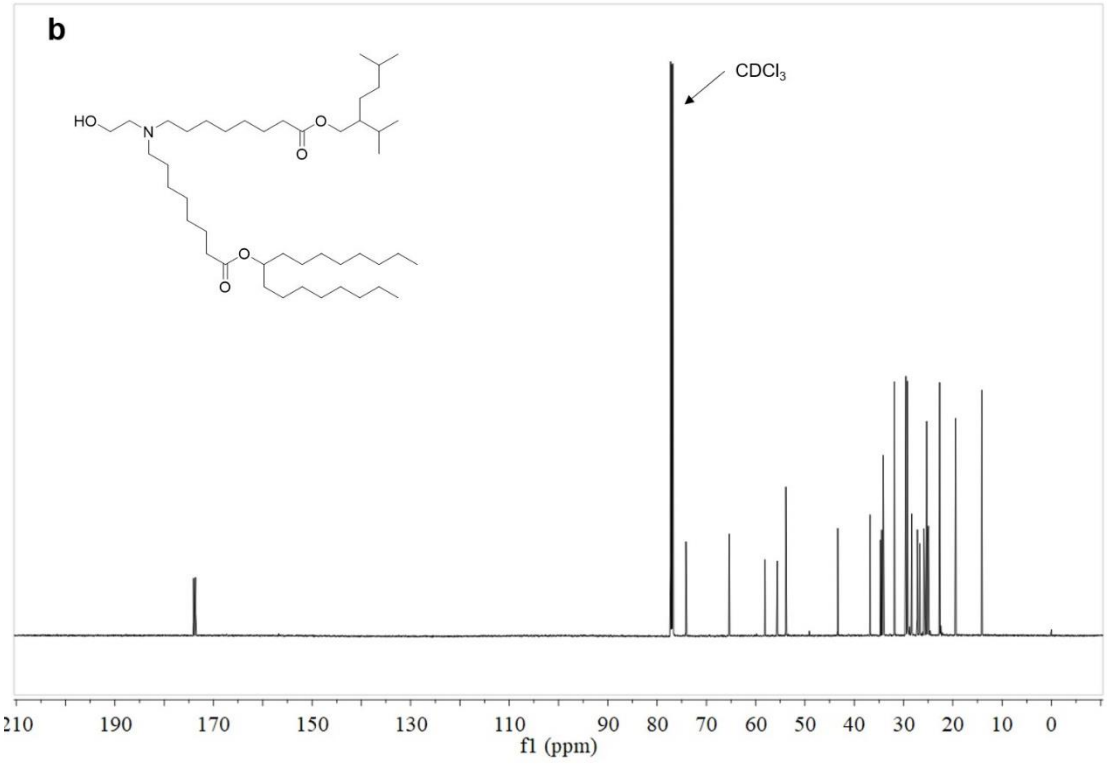
Reactive formula:

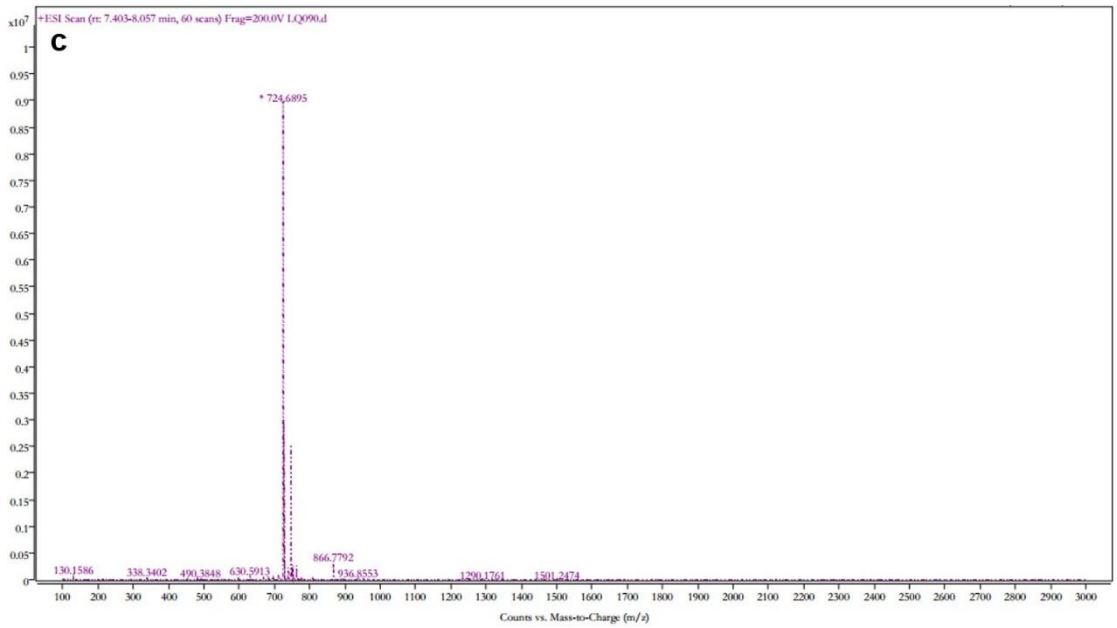


Operation process:

A mixture of LQ090-3 (1 g, 2.3 mmol), LQ090-1 (1 g, 2.75 mmol), potassium carbonate (640 mg, 4.6 mmol) and potassium iodide (380 mg, 2.3 mmol) in acetonitrile (30 mL) was stirred at 80 ℃ overnight. The crystals were filtered and washed on the filter with ethyl acetate (20 mL). The solvent was then removed in vacuo, and the residue was purified by silica gel chromatography (CH_2Cl_2/ MeOH 10:1, Rf = 0.6) afforded LQ090 (800 mg) in 48% yield as oil.


a
26 (m)
4.85
14 (ddd)
4.01
2 (t)
3.57
5,16 (m)
2.50
3 (t)
2.63
11,22 (dt)
2.28
6,17 (p)
1.61
15 (dtd)
1.76
1.00
2.02
1.86
2.02
3.73
3.95
0.96
4.05
8.22
39.52
16.65
fl (ppm)

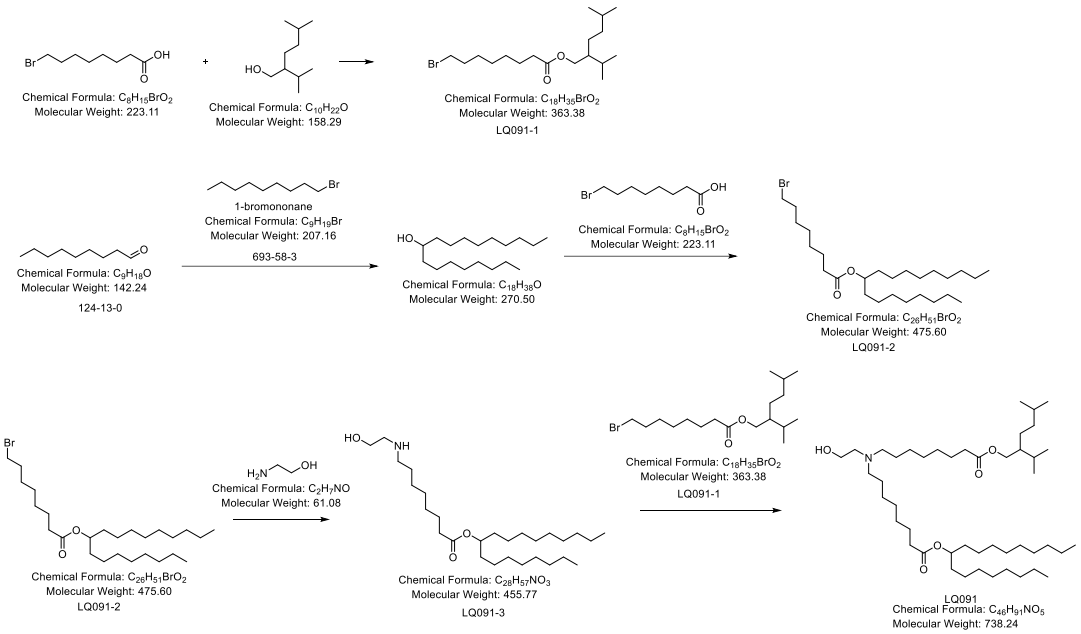

b
CDCl3
fl (ppm)



Supplementary Figure 24. Chemical characterization of LQ090. (a) **^{1}H NMR** (600 MHz, CDCl3) δ 4.89 – 4.82 (m, 1H), 4.01 (ddd, *J* = 26.0, 11.1, 6.0 Hz, 2H), 3.57 (t, *J* = 5.3 Hz, 2H), 2.63 (t, *J* = 5.3 Hz, 2H), 2.52 – 2.48 (m, 3H), 2.28 (dt, *J* = 9.0, 7.6 Hz, 4H), 1.76 (dtd, *J* = 13.8, 6.9, 4.9 Hz, 1H), 1.61 (p, *J* = 7.1 Hz, 4H), 1.52 – 1.43 (m, 8H), 1.36 – 1.14 (m, 39H), 0.90 – 0.84 (m, 16H). (b) **^{13}C NMR** (151 MHz, CDCl3) δ 174.05, 173.65, 74.14, 65.38, 58.09, 55.60, 53.87, 43.34, 36.78, 34.67, 34.43, 34.14, 31.87, 29.54, 29.51, 29.24, 29.20, 29.18, 29.15, 29.13, 28.43, 28.31, 27.22, 27.20, 26.67, 25.85, 25.32, 25.09, 24.95, 22.72, 22.67, 22.49, 19.44, 14.11. (c) **MS** (ESI, m/z) $[M+H]^+$ calculated for $C_{45}H_{89}NO_5$, 724.67; Found, 724.6895.

2.6 2-isopropyl-5-methylhexyl 8-((2-hydroxyethyl)(8-(octadecan-9-yloxy)-8-oxooctyl)amino)octanoate (**LQ091**)

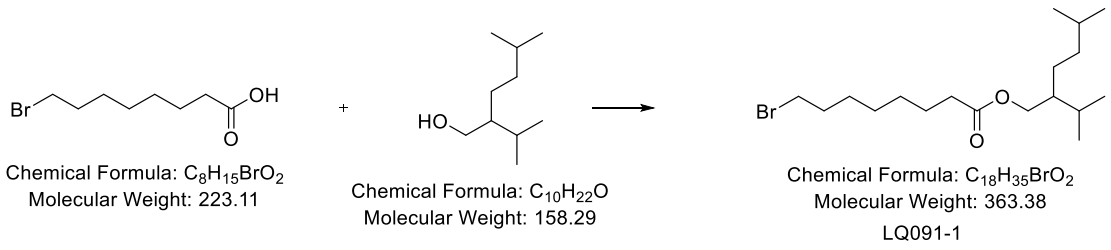
2.6.1 Synthetic route:



2.6.2 Experimental process:

Step 1 Preparation of LQ091-1

Reaction formula:



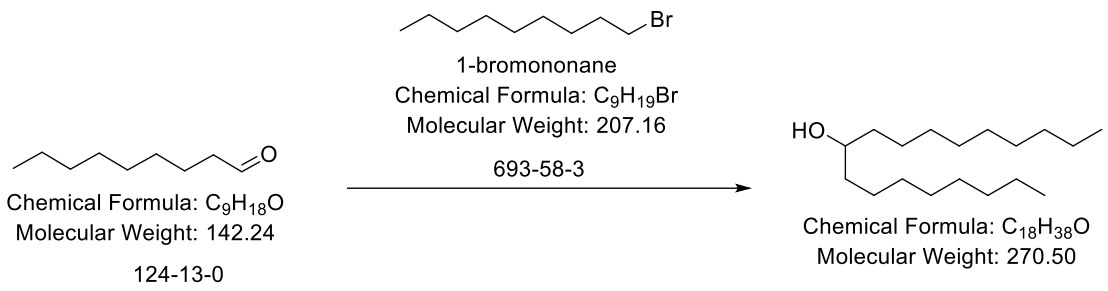
Operation process:

To a solution of 8-bromooctanoic acid (1.56 g, 7 mmol), Dicyclohexylcarbodiimide (DCC, 2.6 g, 12.6 mmol), and 4-Dimethylaminopyridine (DMAP, 80 mg, 0.63 mmol) in DCM (50 mL) was added tetrahydrofuranol (1 g, 6.3 mmol). The resulting mixture was stirred at room temperature overnight. The crystals were filtered and washed on the filter with DCM (25 mL). The solvent was then removed in vacuo, the residue was

purified by silica gel chromatography (hexanes/EtOAc 20:1, Rf = 0.65) afforded LQ091-1 (2 g) in 87% yield as oil.

Step 2 Preparation of 9-Octadecanol

Reaction formula:

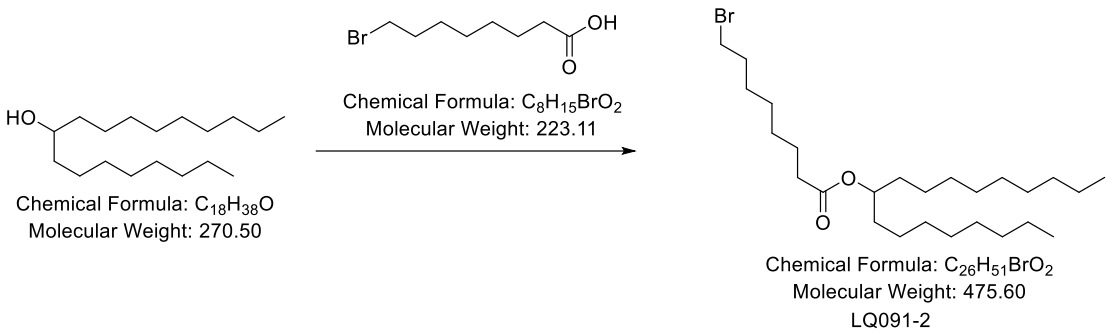


Operation process:

Add magnesium shavings and anhydrous tetrahydrofuran into the reaction flask, protect with nitrogen, heat to 45 ℃, slowly add 1-bromodecane, trigger the Grignard reaction, and the temperature will rise and reflux after the addition is complete. After the addition is complete, keep the reaction at 45 ℃ for 2 hours, then cool to 2-10 ℃, slowly drip nonanal, and stir at room temperature for 12 h, Cool the reaction solution to 2-10 ℃, slowly add 100 mL of 1 N dilute hydrochloric acid, stir for 30 minutes, then add 600 mL of ethyl acetate and 1 L of water extraction, take the organic phase, dry it with anhydrous sodium sulfate, rotary evaporate it, and after column chromatography purification (hexanes/EtOAc 20:1, Rf = 0.3), 81 g of white solid was obtained. Yield 85%.

Step 3 Preparation of LQ091-2

Reaction formula:

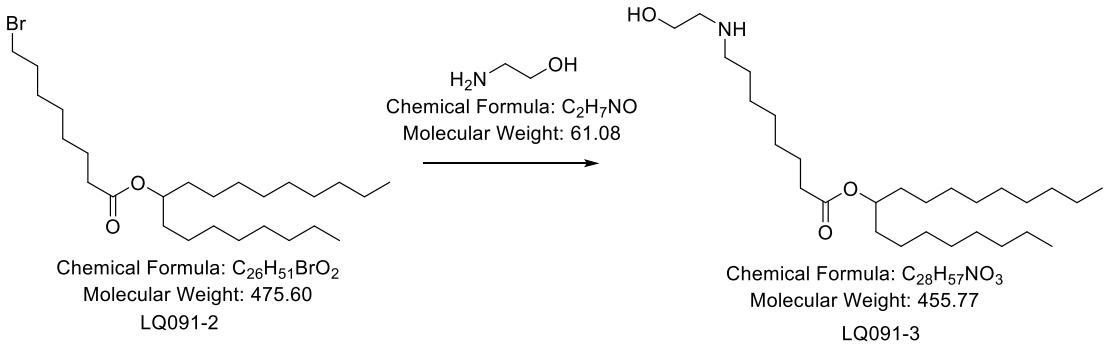


Operation process:

To a solution of 8-bromooctanoic acid (2.45 g, 11 mmol), Dicyclohexylcarbodiimide (DCC, 4.12 g, 20 mmol), and 4-Dimethylaminopyridine (DMAP, 122 mg, 1 mmol) in DCM (50 mL) was added 9-octadecanol (2.7 g, 10 mmol). The resulting mixture was stirred at room temperature overnight. The crystals were filtered and washed on the filter with DCM (25 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography(hexanes/EtOAc 20:1, Rf = 0.6) afforded LQ091-2 (3.8 g) in 80% yield as oil.

Step 4 Preparation of LQ091-3

Reaction formula:



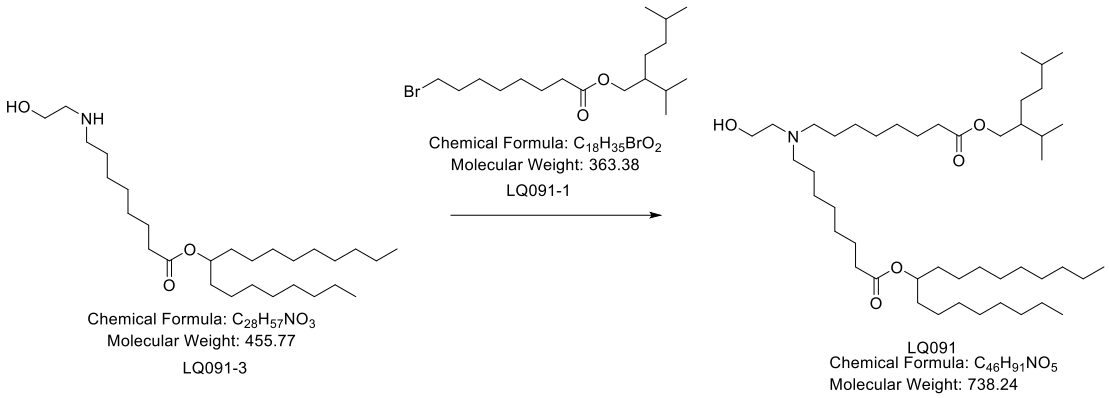
Operation process:

A mixture of LQ091-2 (3.8 g, 8 mmol) and ethanolamine (4.9 g, 80 mmol) in acetonitrile (40 mL) was stirred at 30 ℃ overnight. The reaction mixture was diluted

with 100 mL of ethyl acetate and washed twice with 100 mL of water, and the organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo, the residue was purified by silica gel chromatography(CH_2Cl_2/MeOH 10:1, Rf = 0.4) afforded LQ091-3 (2.8 g) in 79% yield as oil.

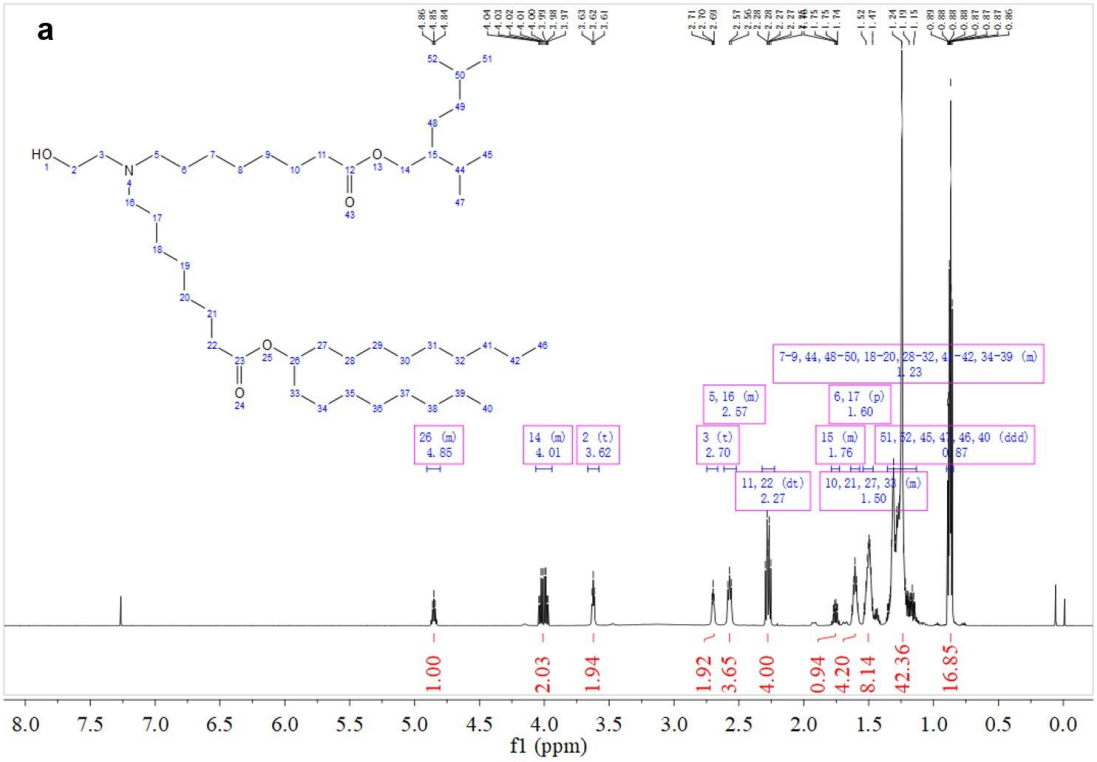
Step 5 Preparation of LQ091

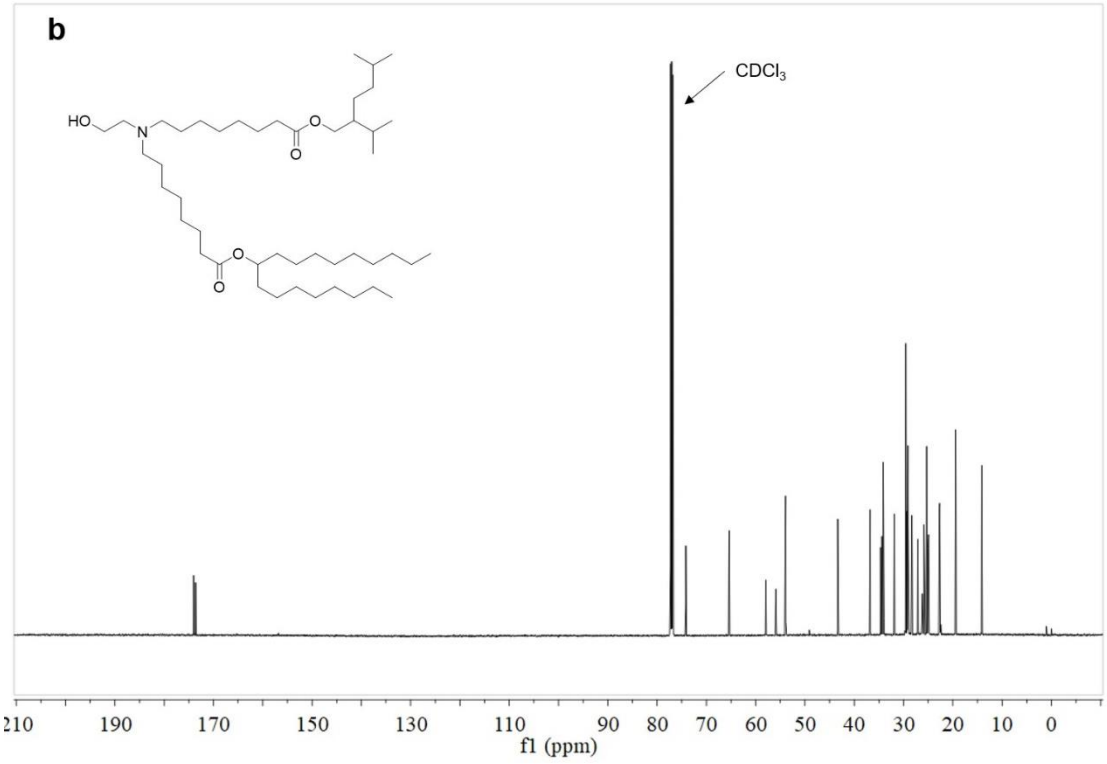
Reaction formula:

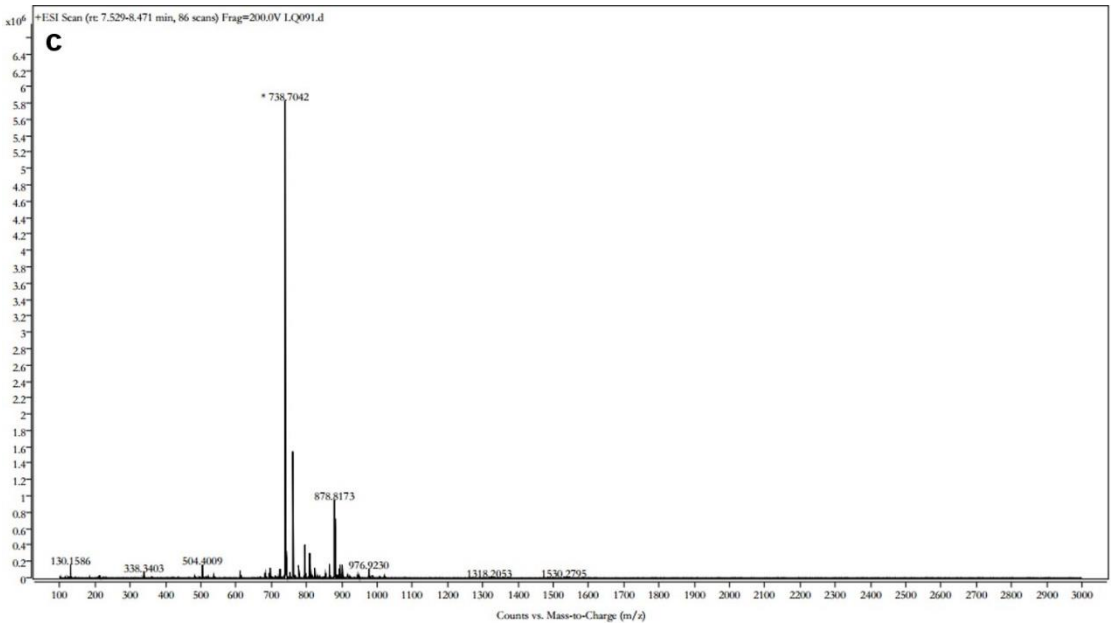


Operating process:

A mixture of LQ091-3 (1 g, 2.3 mmol), LQ091-1(1 g, 2.75 mmol), potassium carbonate (640 mg, 4.6 mmol) and potassium iodide (380 mg, 2.3 mmol) in acetonitrile (30 mL) and was stirred at 80 ℃ overnight. The crystals were filtered and washed on the filter with ethyl acetate (20 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography(CH_2Cl_2/MeOH 10:1, Rf = 0.6) afforded LQ090 (900mg) in 53% yield as oil.


a
26 (m)
4.86
14 (m)
4.01
2 (t)
3.62
3 (t)
2.70
5, 16 (m)
2.57
11, 22 (dt)
2.27
6, 17 (p)
1.60
15 (m)
1.76
10, 21, 27, 33 (m)
1.50
1.00
2.03
1.94
1.92
3.65
4.00
0.94
4.20
8.14
42.36
16.85
f1 (ppm)

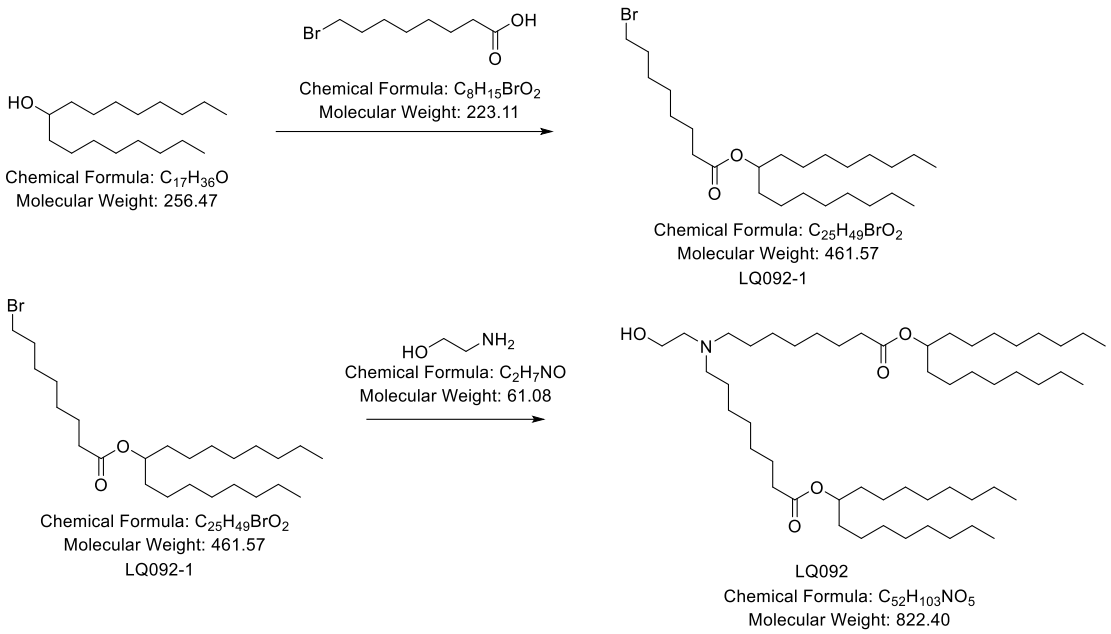

b
CDCl3
f1 (ppm)



Supplementary Figure 25. Chemical characterization of LQ091. (a) **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.90 – 4.80 (m, 1H), 4.06 – 3.94 (m, 2H), 3.62 (t, *J* = 5.1 Hz, 2H), 2.70 (t, *J* = 5.0 Hz, 2H), 2.62 – 2.52 (m, 4H), 2.27 (dt, *J* = 9.3, 7.6 Hz, 4H), 1.79 – 1.72 (m, 1H), 1.60 (p, *J* = 7.1 Hz, 4H), 1.54 – 1.47 (m, 8H), 1.36 – 1.13 (m, 42H), 0.87 (ddd, *J* = 9.6, 6.8, 4.8 Hz, 16H). (b) **^{13}C NMR** (151 MHz, $CDCl_3$) δ 174.03, 173.62, 74.17, 65.39, 53.93, 43.34, 36.78, 34.64, 34.41, 34.14, 31.90, 31.87, 29.55, 29.54, 29.51, 29.31, 29.24, 29.13, 29.11, 29.09, 28.43, 28.31, 27.13, 27.11, 25.84, 25.32, 25.05, 24.91, 22.72, 22.68, 22.67, 22.49, 19.43, 14.12. (c) **MS** (ESI, m/z) $[M+H]^+$ calculated for $C_{46}H_{91}NO_5$, 738.69; Found, 738.7042.

2.7 di(heptadecan-9-yl) 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate (**LQ092**)

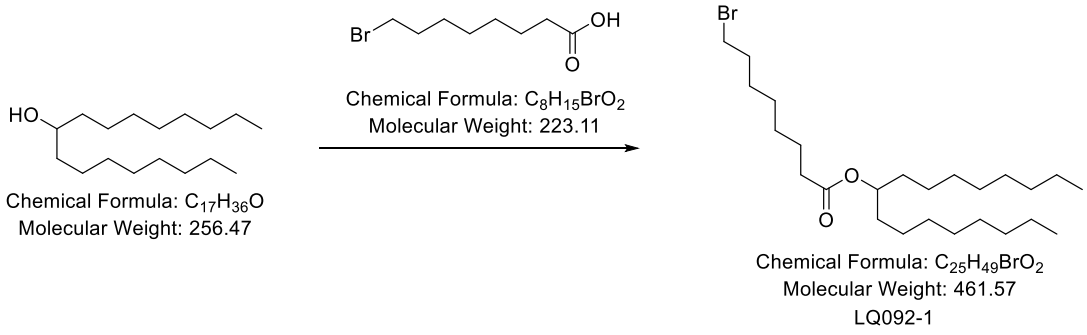
2.7.1 Synthetic route:



2.7.2 Experimental process

Step 1 Preparation of LQ092-1

Reaction equation:



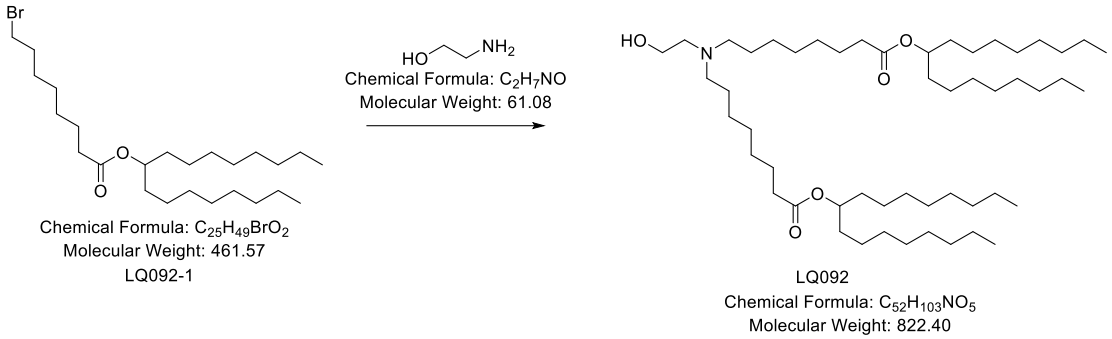
Operating process:

To a solution of 8-bromooctanoic acid (2.45 g, 11 mmol), Dicyclohexylcarbodiimide (DCC, 4.12 g, 20 mmol), and 4-Dimethylaminopyridine (DMAP, 122 mg, 1 mmol) in DCM (50 mL) was added 9-heptadecanol (2.6 g, 10 mmol). The resulting mixture was stirred at room temperature overnight. The crystals were filtered and washed on the filter with DCM (25 mL). The solvent was then removed in vacuo, the residue was

purified by silica gel chromatography (hexanes/EtOAc 20:1, Rf = 0.6) afforded LQ092-1 (3.6 g) in 78% yield as oil.

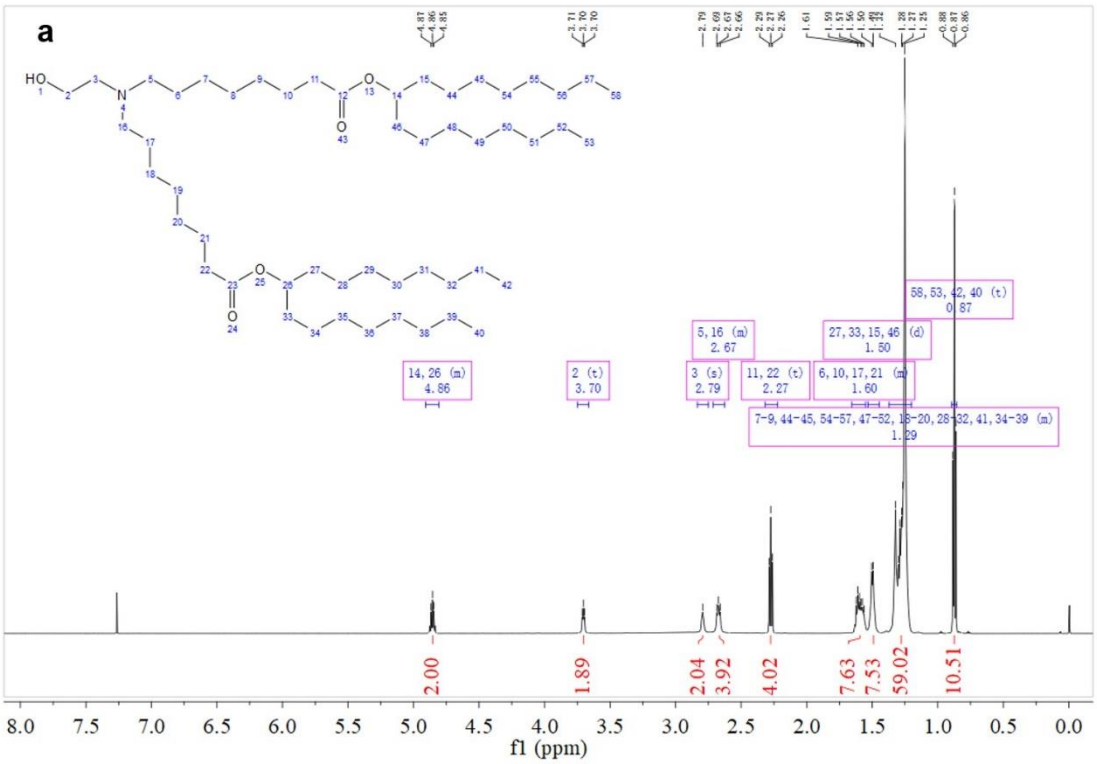
Step 2 Preparation of LQ092

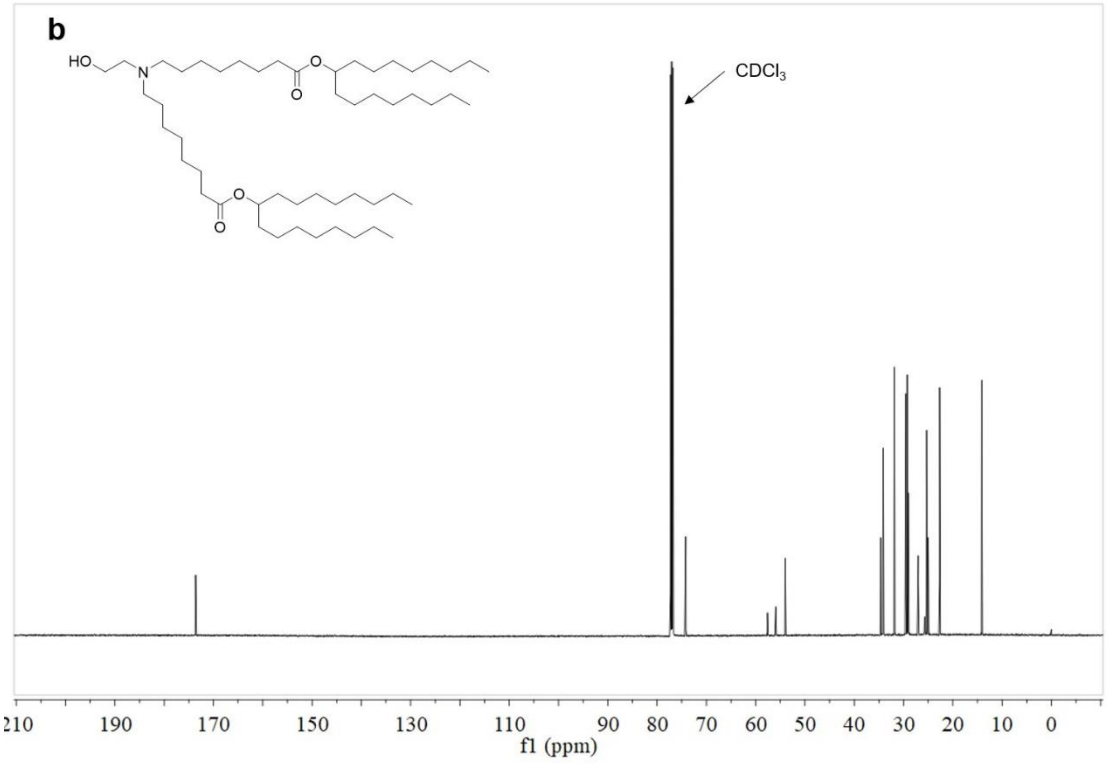
Reaction equation:

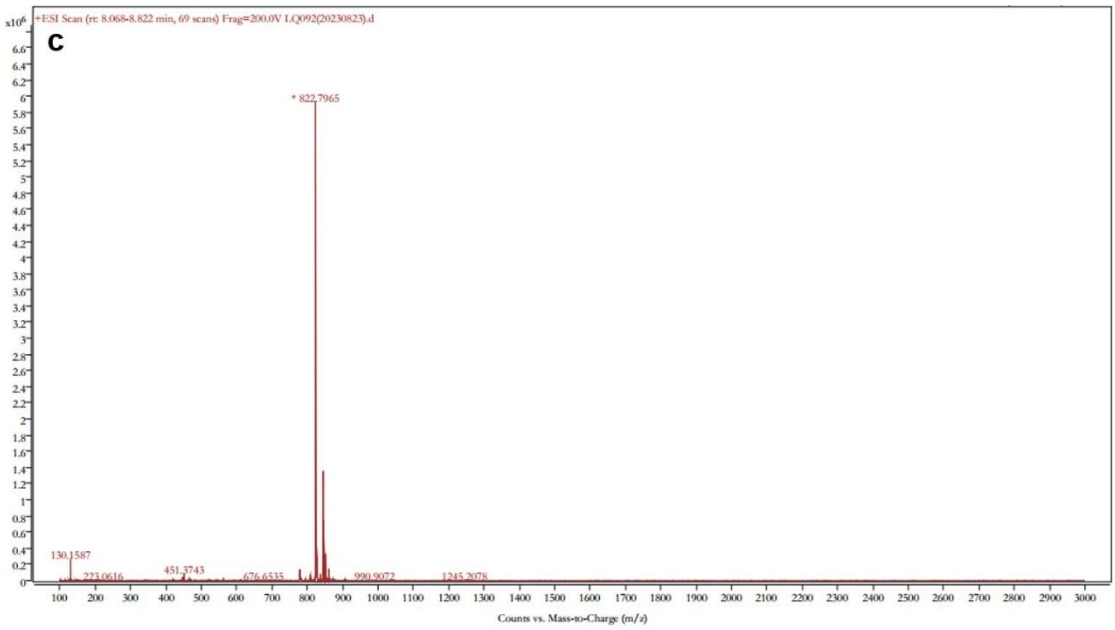


Operating process:

A mixture of LQ092-1 (2 g, 4.6 mmol), ethanolamine (112 mg, 1.85 mmol), potassium carbonate (1 g,7.4 mmol) and potassium iodide(620 mg, 3.7 mmol) in acetonitrile (30 mL) and was stirred at 80 ℃ overnight. The crystals were filtered and washed on the filter with ethyl acetate (20 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 10:1, Rf = 0.6) afforded LQ092 (800 mg) in 53% yield as oil.


a
14, 26 (m)
4.86
2 (t)
3.70
3 (s)
2.79
5, 16 (m)
2.67
11, 22 (t)
2.27
6, 10, 17, 21 (m)
1.60
27, 33, 15, 46 (d)
1.50
58, 53, 42, 40 (t)
0.87
7-9, 44-45, 54-57, 47-52, 18-20, 28-32, 41, 34-39 (m)
1.29
2.00
1.89
2.04
3.92
4.02
7.63
7.53
59.02
10.51
f1 (ppm)

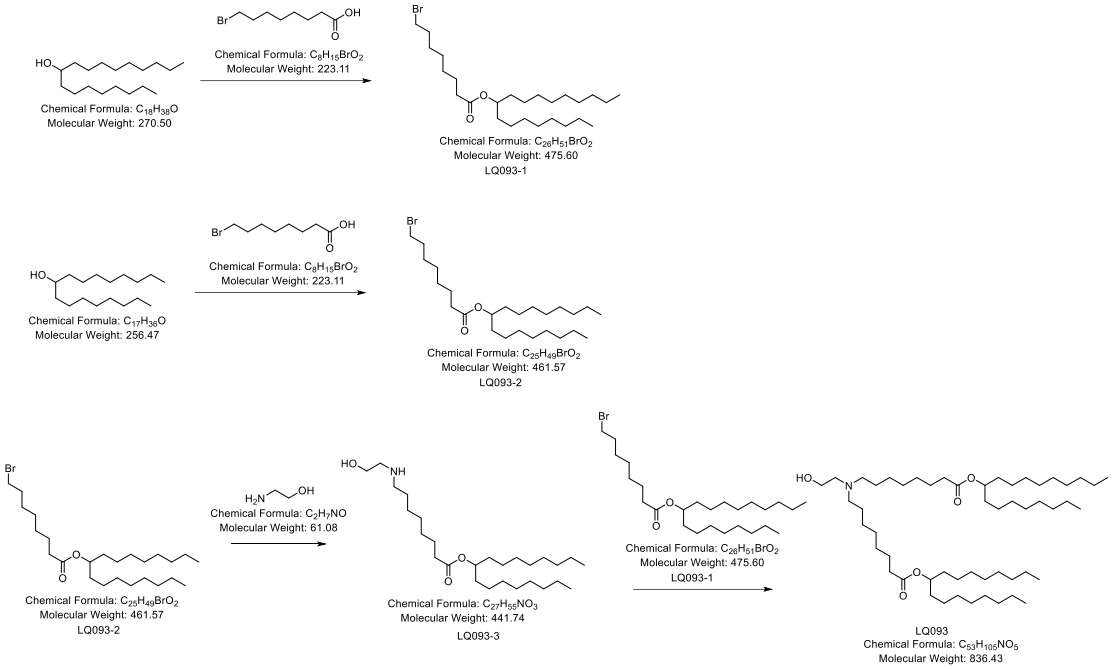

b
CDCl3
f1 (ppm)



Supplementary Figure 26. Chemical characterization of LQ092. (a) **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.91 – 4.81 (m, 2H), 3.70 (t, *J* = 4.9 Hz, 2H), 2.79 (s, 2H), 2.71 – 2.63 (m, 4H), 2.27 (t, *J* = 7.5 Hz, 4H), 1.65 – 1.55 (m, 8H), 1.50 (d, *J* = 6.0 Hz, 8H), 1.37 – 1.20 (m, 59H), 0.87 (t, *J* = 7.0 Hz, 11H). (b) **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.62, 74.19, 57.60, 55.90, 54.01, 34.62, 34.14, 31.87, 29.54, 29.51, 29.25, 29.06, 27.03, 25.33, 25.01, 22.67, 14.12. (c) **MS** (ESI, m/z) $[M+H]^+$ calculated for $C_{52}H_{103}NO_5$, 822.78; Found, 822.7965.

2.8 heptadecan-9-yl 8-((2-hydroxyethyl)(8-(octadecan-9-yloxy)-8-oxooctyl)amino)octanoate (**LQ093**)

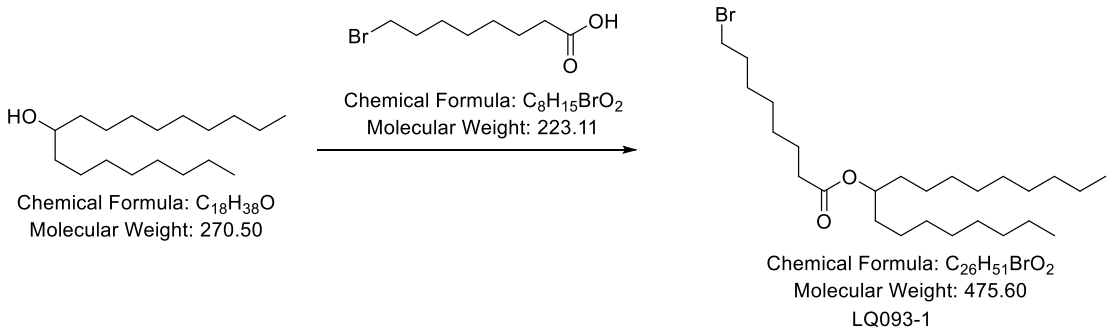
2.8.1 Synthetic route



2.8.2 Experimental process

Step 1 Preparation of LQ093-1

Reaction formula:



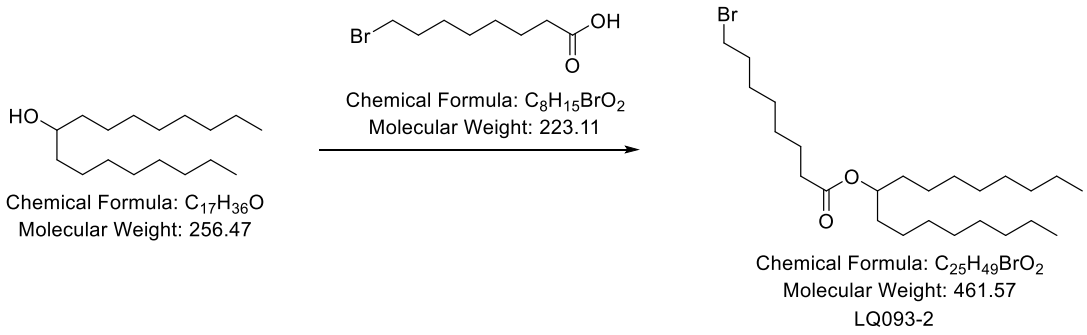
Operational process:

To a solution of 8-bromooctanoic acid (2.45 g, 11 mmol), Dicyclohexylcarbodiimide (DCC, 4.12 g, 20 mmol), and 4-Dimethylaminopyridine (DMAP, 122 mg, 1 mmol) in DCM(50 mL) was added 9-octadecanol (2.7 g, 10 mmol). The resulting mixture was stirred at room temperature overnight. The crystals were filtered and washed on the

filter with DCM (25 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (hexanes/EtOAc 20:1, Rf = 0.6) afforded LQ093-1 (3.8 g) in 80% yield as oil.

Step 2 Preparation of LQ093-2

Reaction formula:

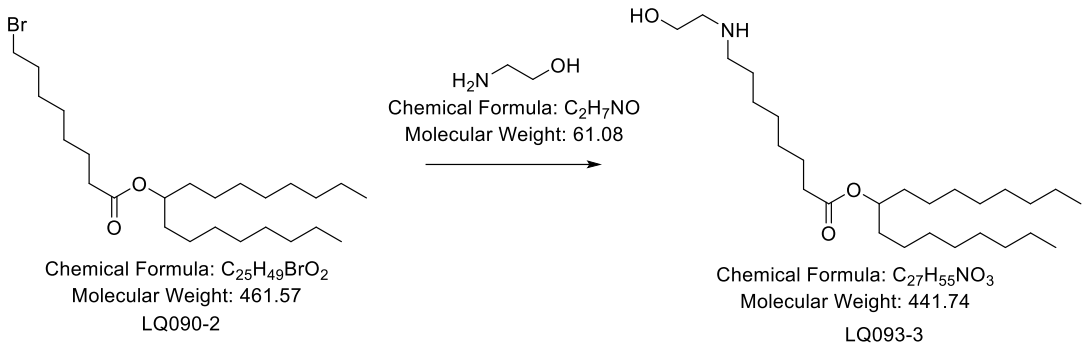


Operational process:

To a solution of 8-bromooctanoic acid (2.45 g, 11 mmol), Dicyclohexylcarbodiimide (DCC, 4.12 g, 20 mmol), and 4-Dimethylaminopyridine (DMAP, 122 mg, 1 mmol) in DCM (50 mL) was added 9-heptadecanol (2.6 g, 10 mmol). The resulting mixture was stirred at room temperature overnight. The crystals were filtered and washed on the filter with DCM (25 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (hexanes/EtOAc 20:1, Rf = 0.6) afforded LQ093-2 (3.6 g) in 78% yield as oil.

Step 3 Preparation of LQ093-3

Reaction formula:

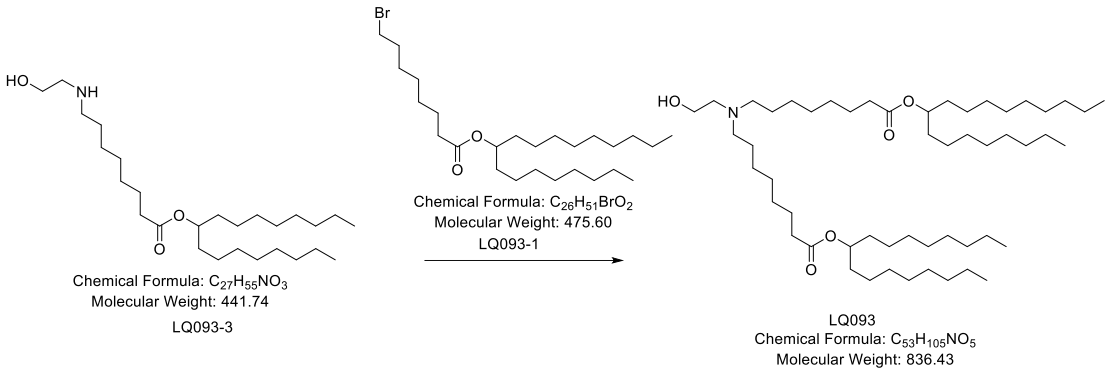


Operational process:

A mixture of LQ093-2 (3.6 g, 8 mmol) and ethanolamine(4.9 g,80 mmol) in acetonitrile (40 mL) was stirred at 30℃ overnight. The reaction mixture was diluted with 100 mL of ethyl acetate, washed twice with 100 mL of water, and the organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 10:1, Rf = 0.4) afforded LQ093-3 (2.8 g) in 79% yield as oil.

Step 4 Preparation of LQ093

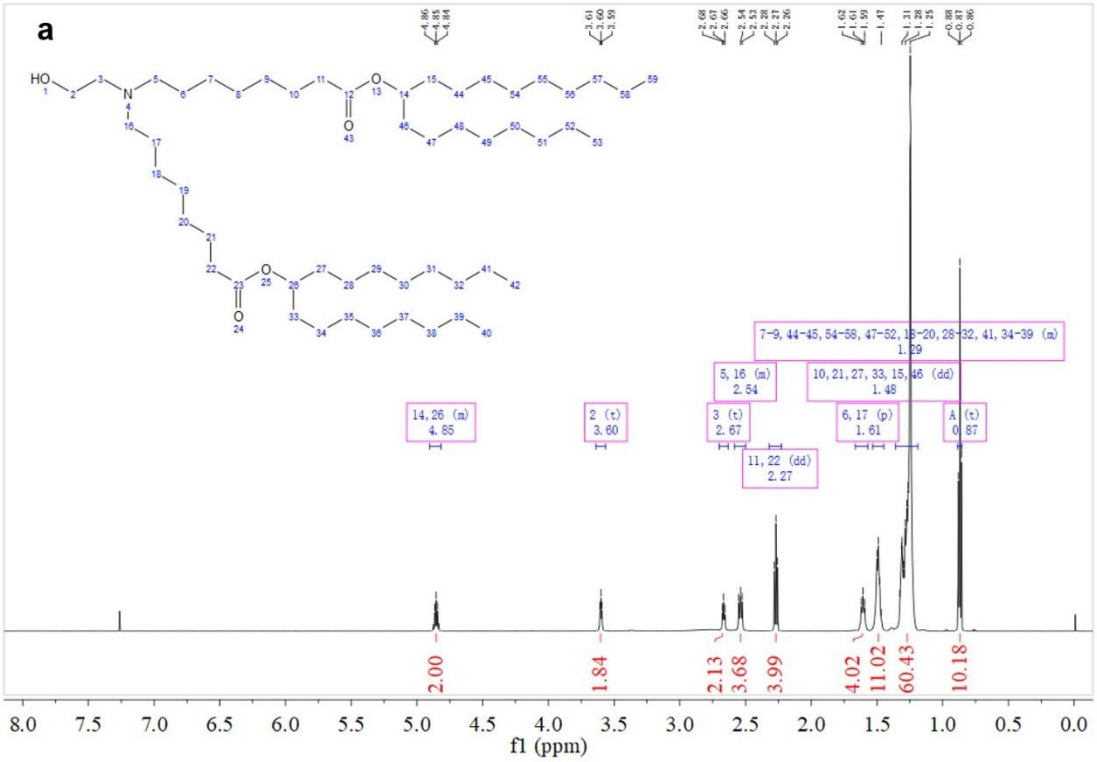
Reactants:

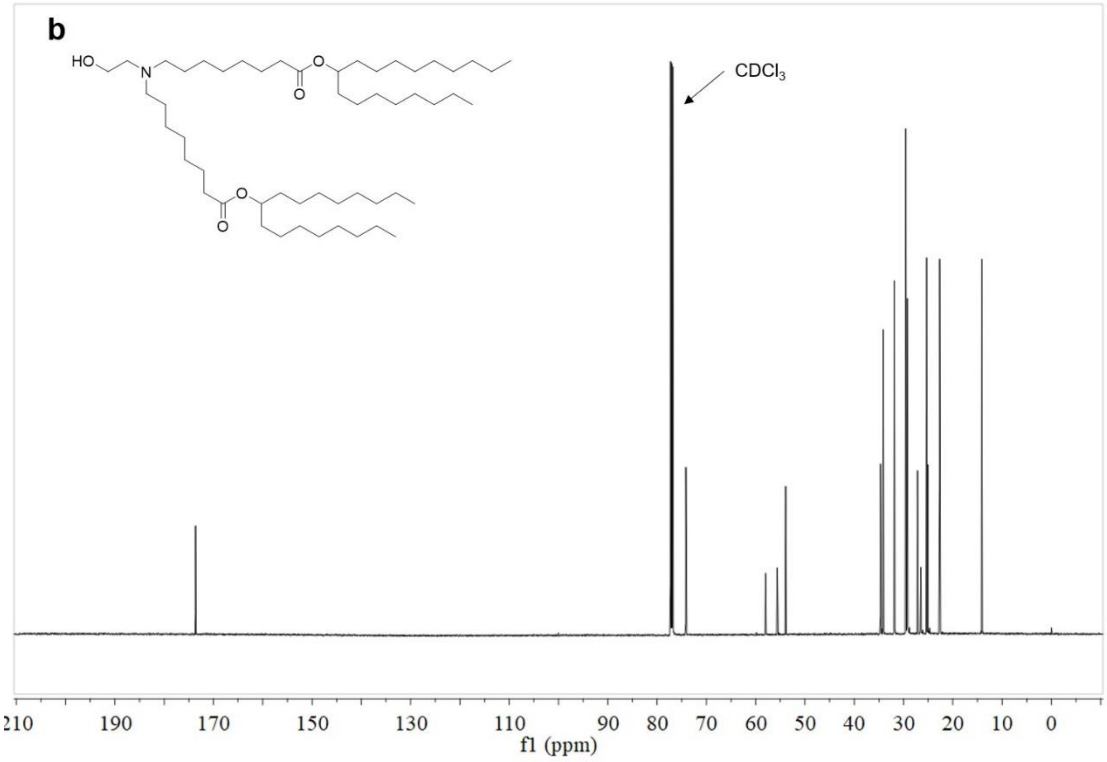


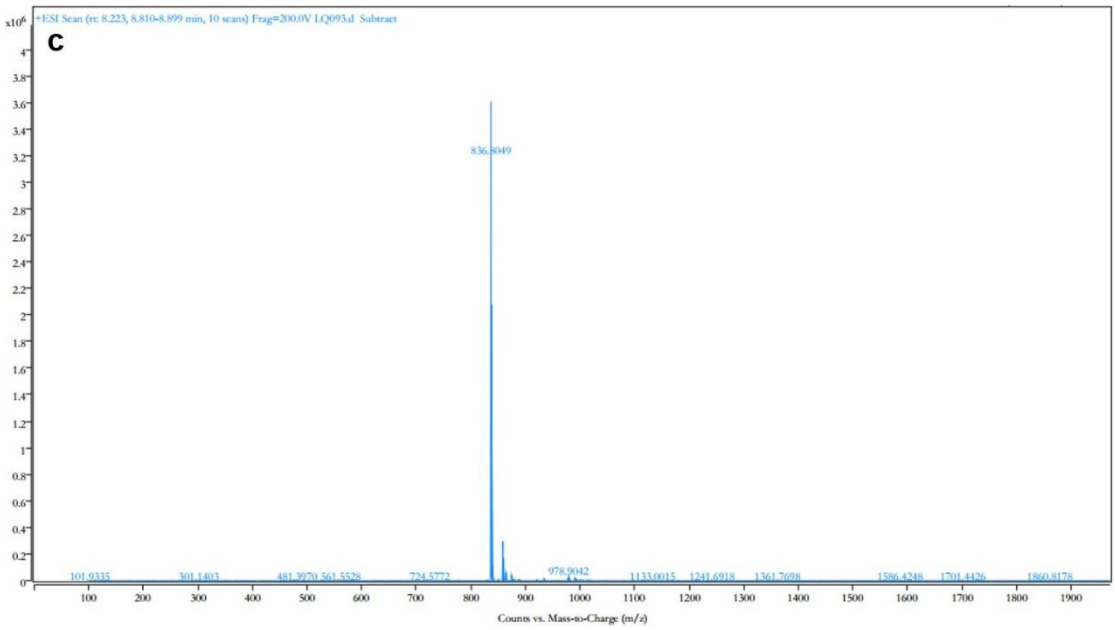
Operational process:

A mixture of LQ093-3 (1 g, 2.3 mmol), LQ093-1(1.3 g, 2.75 mmol), potassium carbonate (640 mg,4.6 mmol) and potassium iodide (380 mg, 2.3 mmol) in acetonitrile (30 mL) and was stirred at 80 ℃ overnight. The crystals were filtered and washed on

the filter with ethyl acetate (20 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography(CH_2Cl_2/MeOH 10:1, Rf = 0.6) afforded LQ093 (900 mg) in 47% yield as oil.



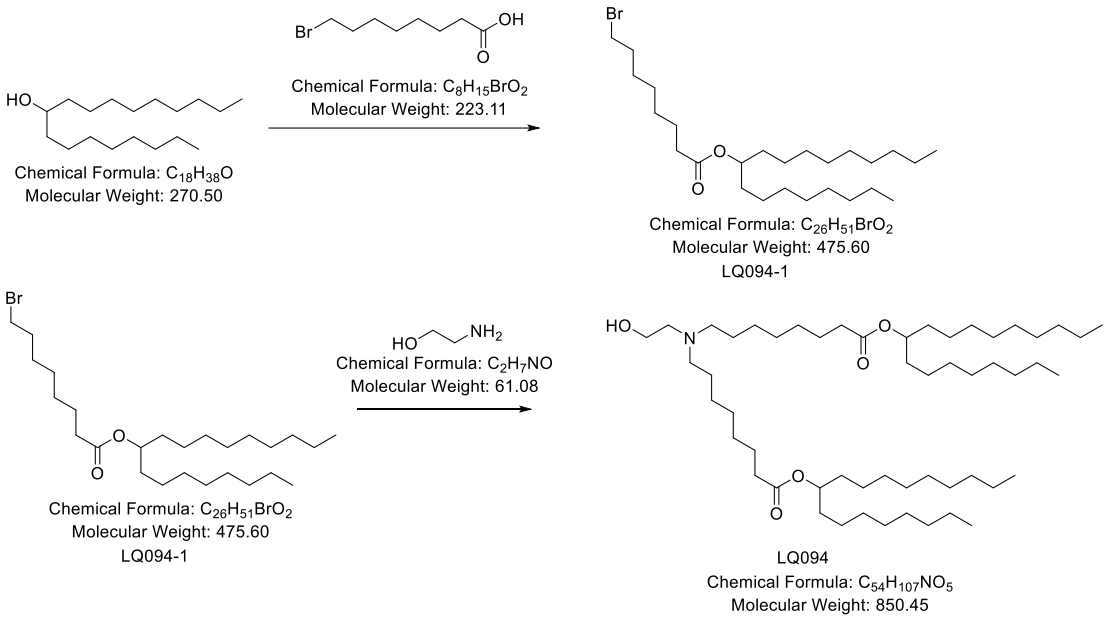




Supplementary Figure 27. Chemical characterization of LQ093. (a) **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.90 – 4.82 (m, 2H), 3.60 (t, *J* = 5.2 Hz, 2H), 2.67 (t, *J* = 5.2 Hz, 2H), 2.59 – 2.50 (m, 4H), 2.27 (dd, *J* = 14.1, 6.6 Hz, 4H), 1.61 (p, *J* = 7.3 Hz, 4H), 1.48 (dd, *J* = 13.4, 7.2 Hz, 11H), 1.36 – 1.19 (m, 60H), 0.87 (t, *J* = 9.1, 5.0 Hz, 10H). (b) **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.63, 74.15, 55.64, 53.91, 34.66, 34.14, 31.90, 31.87, 29.55, 29.54, 29.51, 29.32, 29.24, 29.17, 29.13, 27.18, 26.47, 25.32, 25.07, 22.68, 22.67, 14.11. (c) **MS** (ESI, m/z) $[M+H]^+$ calculated for $C_{53}H_{105}NO_5$, 836.80; Found, 836.8049.

2.9 di(octadecan-9-yl) 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate (**LQ094**)

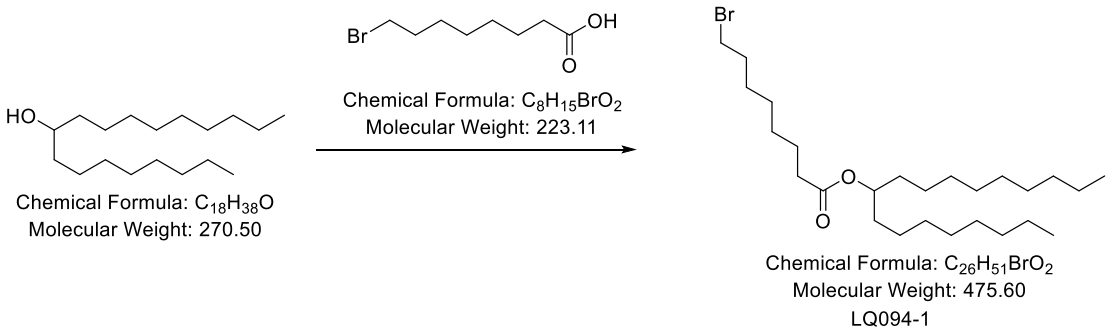
2.9.1 Synthetic route



2.9.2 Experimental process

Step 1 Preparation of LQ094-1

Reaction formula:



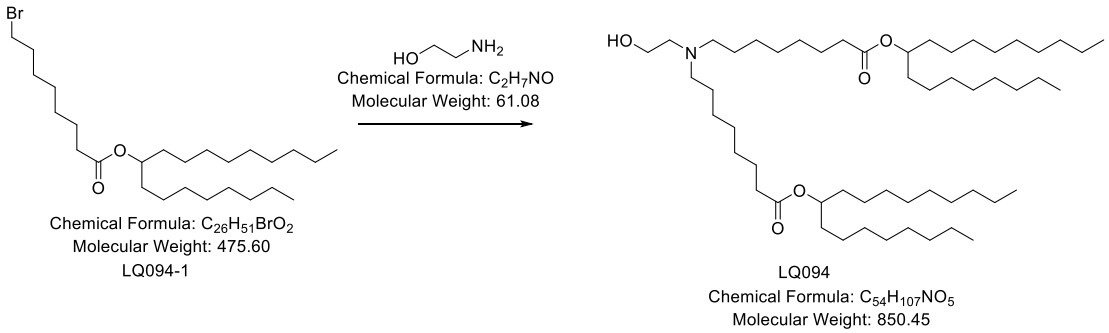
Operating process:

To a solution of 8-bromooctanoic acid (2.45 g, 11 mmol), Dicyclohexylcarbodiimide (DCC, 4.12 g, 20 mmol), and 4-Dimethylaminopyridine (DMAP, 122 mg, 1 mmol) in DCM (50 mL) was added 9-octadecanol (2.7 g, 10 mmol). The resulting mixture was stirred at room temperature overnight. The crystals were filtered and washed on the filter with DCM (25 mL). The solvent was then removed in vacuo, the residue was

purified by silica gel chromatography (hexanes/EtOAc 20:1, Rf = 0.6) afforded LQ094-1 (3.8 g) in 80% yield as oil.

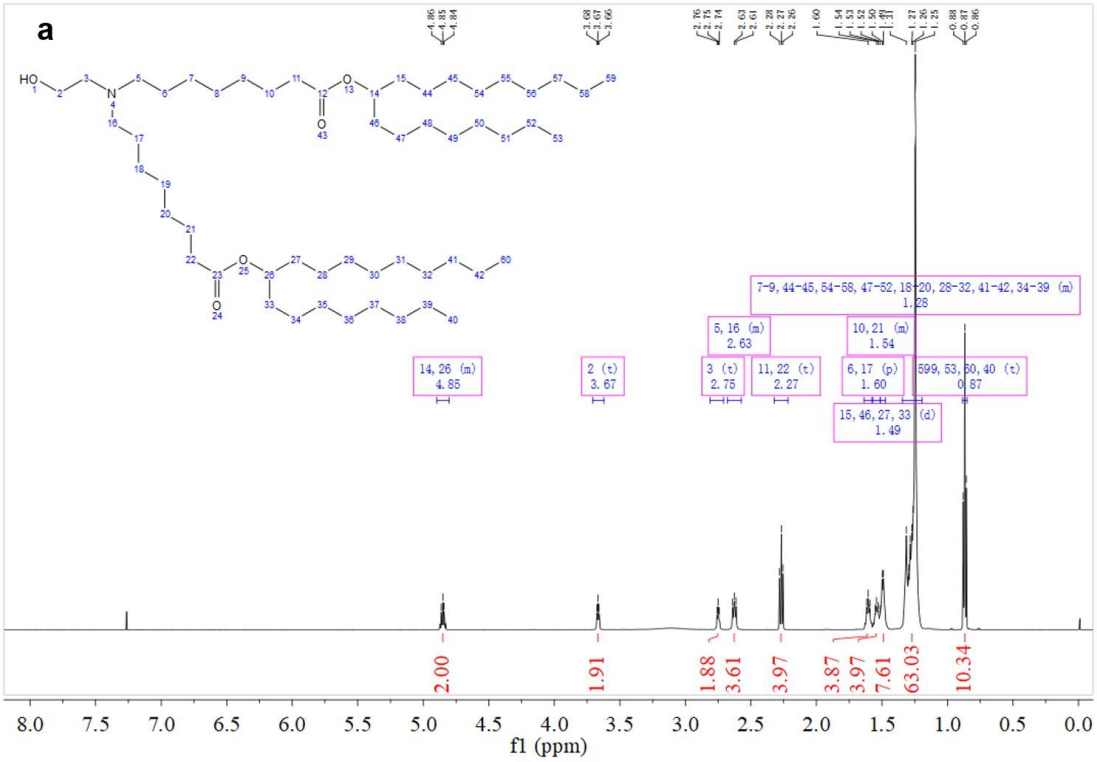
Step 2 Preparation of LQ094

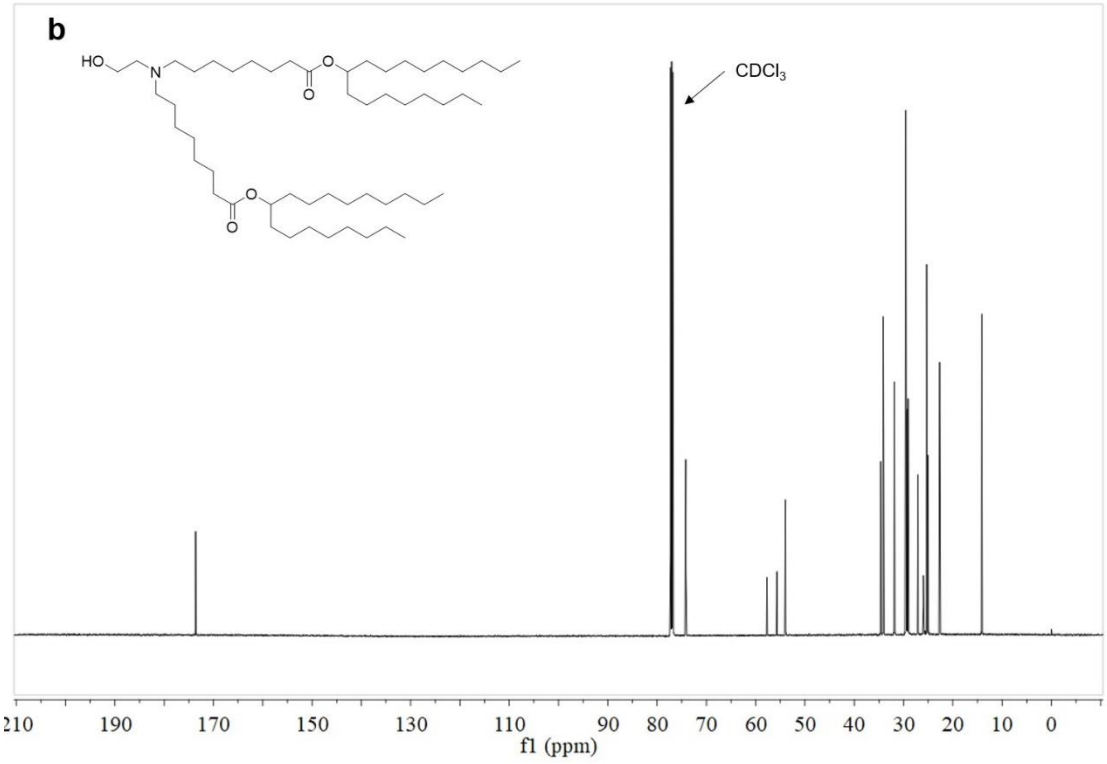
Reaction formula:

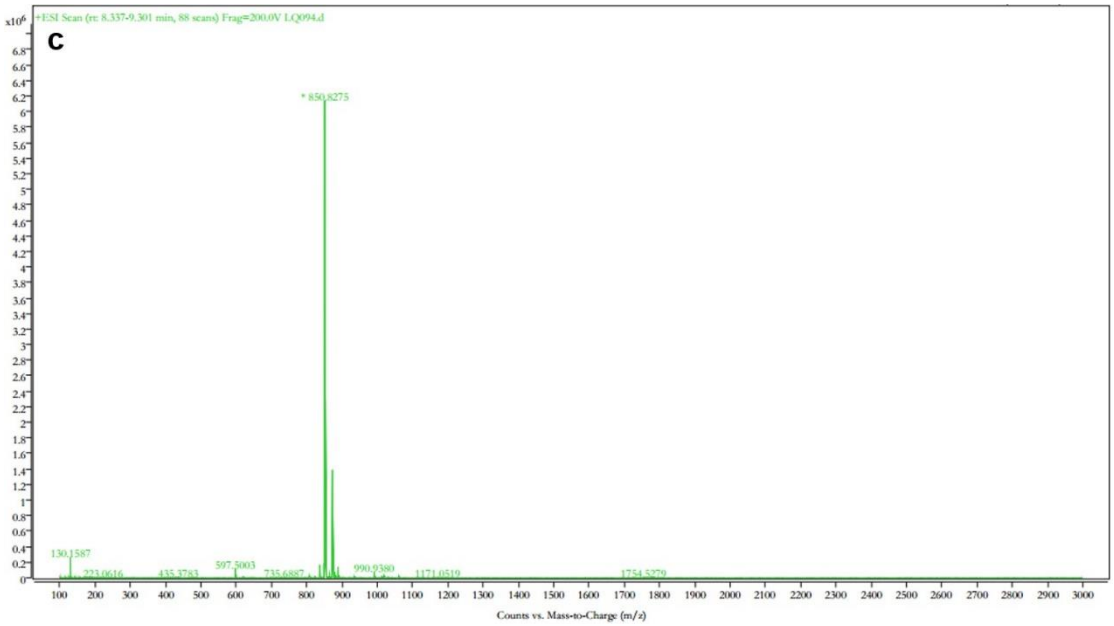


Operating process:

A mixture of LQ094-1 (2 g, 4.2 mmol), ethanolamine (103 mg, 1.68 mmol), potassium carbonate (1 g, 7.4 mmol) and potassium iodide (620 mg,3.7 mmol) in acetonitrile (30 mL) was stirred at 80 ℃ overnight. The crystals were filtered and washed on the filter with ethyl acetate (20 mL). The solvent was then removed in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 10:1, Rf = 0.6) afforded LQ094 (700 mg) in 49% yield as oil.


a
7-9, 44-45, 54-58, 47-52, 18-20, 28-32, 41-42, 34-39 (m)
1.28
5, 16 (m)
2.63
10, 21 (m)
1.54
14, 26 (m)
4.85
2 (t)
3.67
3 (t)
2.75
11, 22 (t)
2.27
6, 17 (p)
1.60
599, 53, 60, 40 (t)
0.87
15, 46, 27, 33 (d)
1.49
2.00
1.91
1.88
3.61
3.97
3.87
3.97
7.61
63.03
10.34
f1 (ppm)


b
CDCl3
f1 (ppm)



Supplementary Figure 28. Chemical characterization of LQ094. (a) **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.90 – 4.80 (m, 2H), 3.67 (t, *J* = 5.2 Hz, 2H), 2.75 (t, *J* = 5.1 Hz, 2H), 2.68 – 2.57 (m, 4H), 2.27 (t, *J* = 7.5 Hz, 4H), 1.60 (p, *J* = 7.3 Hz, 4H), 1.57 – 1.52 (m, 4H), 1.49 (d, *J* = 6.0 Hz, 8H), 1.34 – 1.19 (m, 63H), 0.87 (t, *J* = 7.0 Hz, 10H). (b) **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.62, 74.18, 53.95, 34.63, 34.13, 31.90, 31.87, 29.55, 29.54, 29.51, 29.31, 29.24, 29.09, 27.08, 25.32, 25.03, 22.68, 22.67, 14.12. (c) **MS** (ESI, m/z) $[M+H]^+$ calculated for $C_{54}H_{107}NO_5$, 850.81; Found, 850.8275.

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11. Qiu, M. *et al.* Lipid nanoparticle-mediated codelivery of Cas9 mRNA and single-guide RNA achieves liver-specific in vivo genome editing of Angptl3. *Proceedings of the National Academy of Sciences* **118**, e2020401118 (2021).