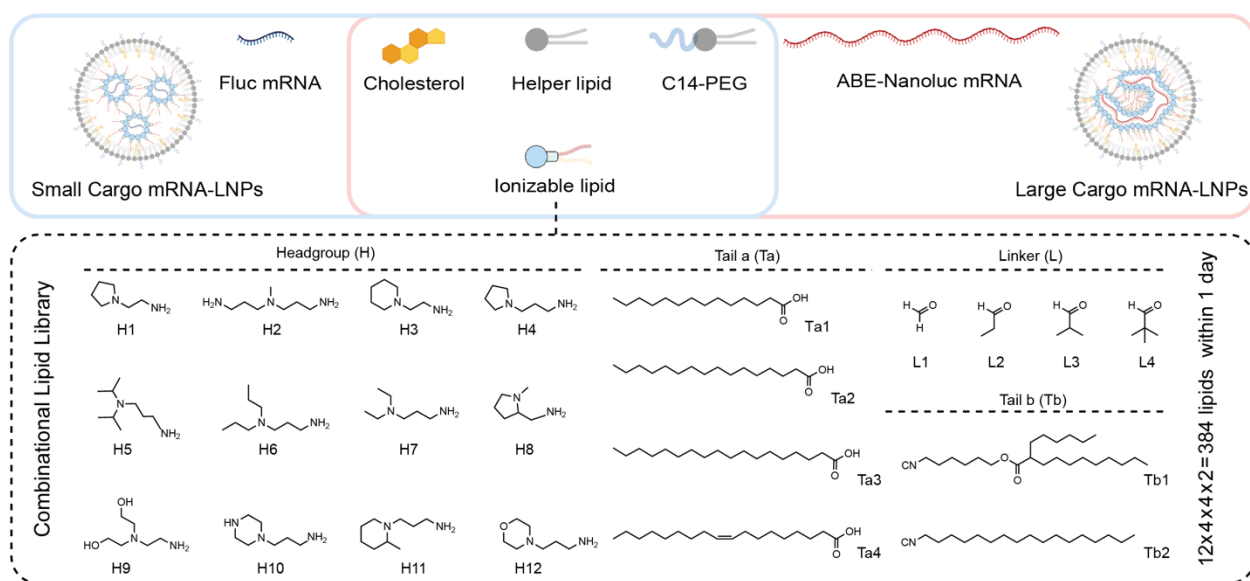


Lipid nanoparticles optimized for large RNA cargo and tissue targeting enhance in vivo genome editing

In the format provided by the
authors and unedited

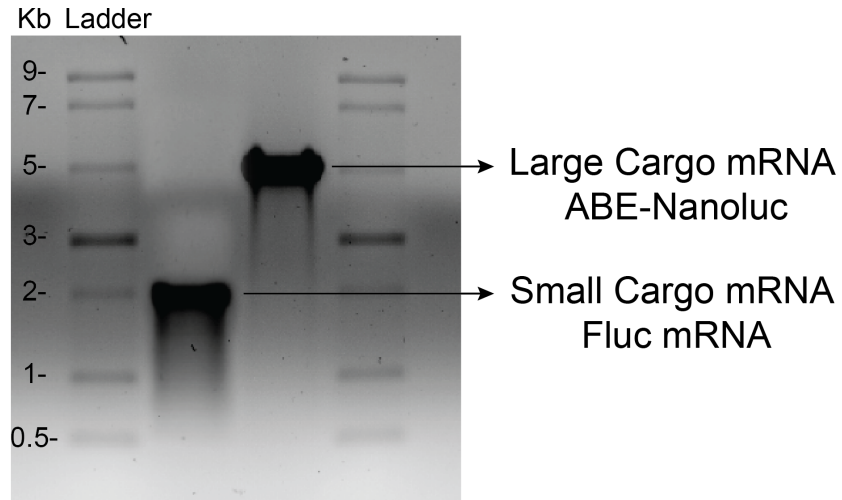
Table-of-contents

Supplementary Fig. 1-Fig.101	2
Table 1-5	77



Supplementary Fig.1 | Combinatorial design of ionizable lipid libraries for small- and large-cargo mRNA-LNPs.

Schematic illustration of the formulation and design strategy for mRNA lipid nanoparticles (LNPs). Each LNP comprises an ionizable lipid, cholesterol, helper lipid, and C14-PEG, encapsulating either a small-cargo mRNA (Firefly luciferase, Fluc) or a large-cargo mRNA (adenine base editor, ABE-NanoLuc). The ionizable lipid library was generated through a combinatorial approach using 12 headgroups (H), 4 tails a (Ta), 2 tails b (Tb), and 4 linkers (L), yielding a total of $12 \times 4 \times 2 \times 4 = 384$ unique lipids. This modular design enables systematic optimization of lipid structures for efficient delivery of mRNA cargos of different sizes.

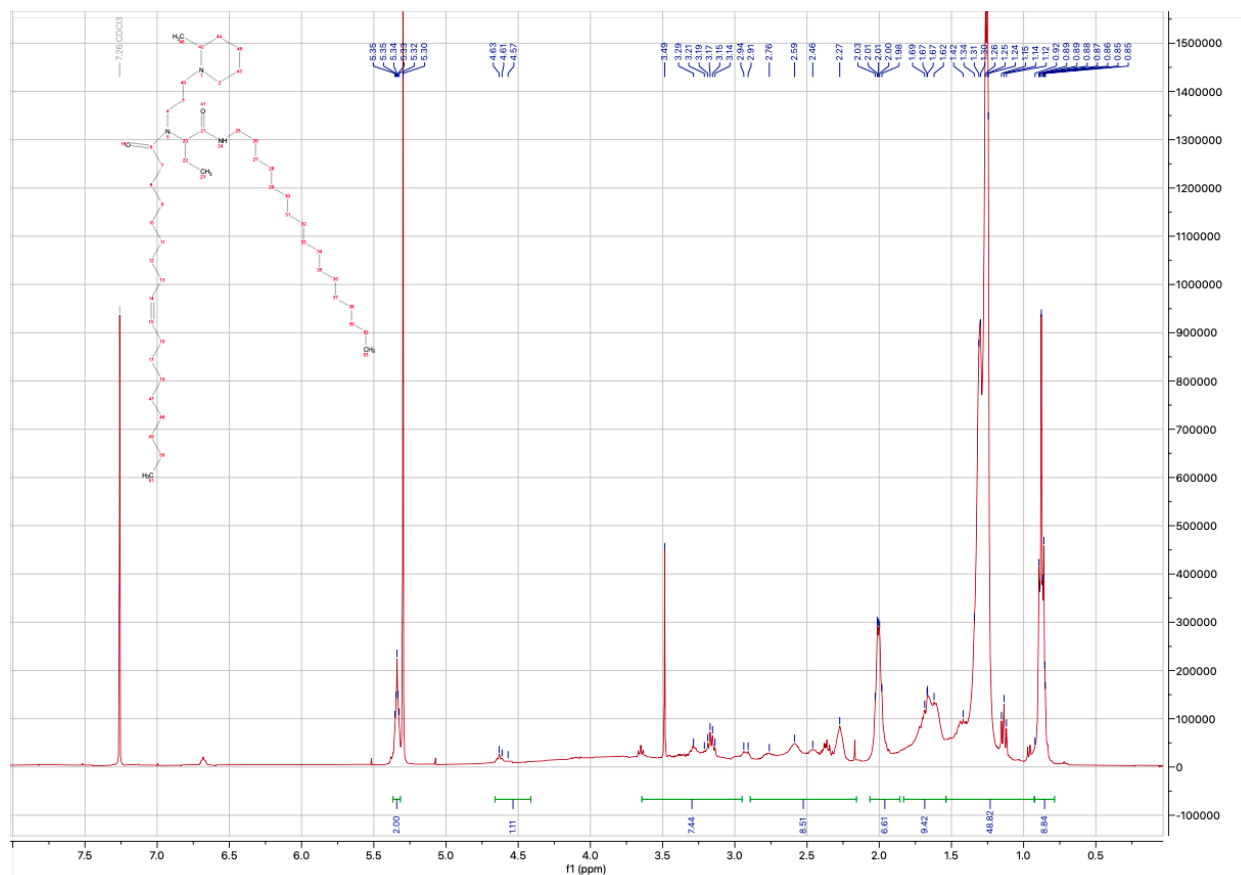


Supplementary Fig. 2 | Agarose gel electrophoresis analysis of small- and large-cargo mRNAs.

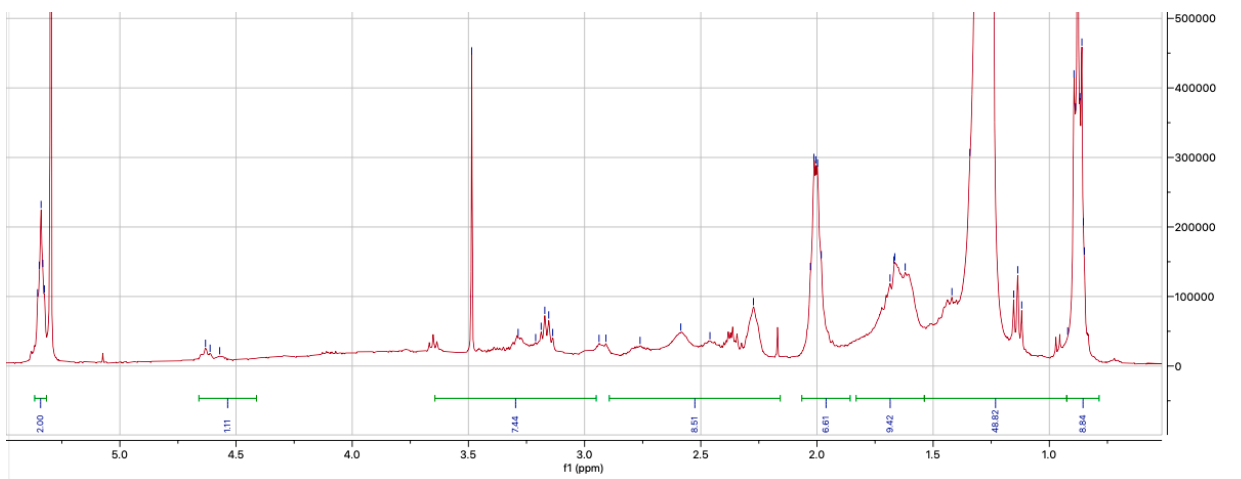
Representative gel image showing the size difference between the small-cargo Fluc mRNA (~1.7 kb) and the large-cargo ABE-Nanoluc mRNA (~5.7 kb). Both mRNAs were synthesized by in vitro transcription and exhibit distinct single bands of expected sizes.

SC-Lipid-1 (C₄₈H₉₃N₃O₂; MW: 744.29):

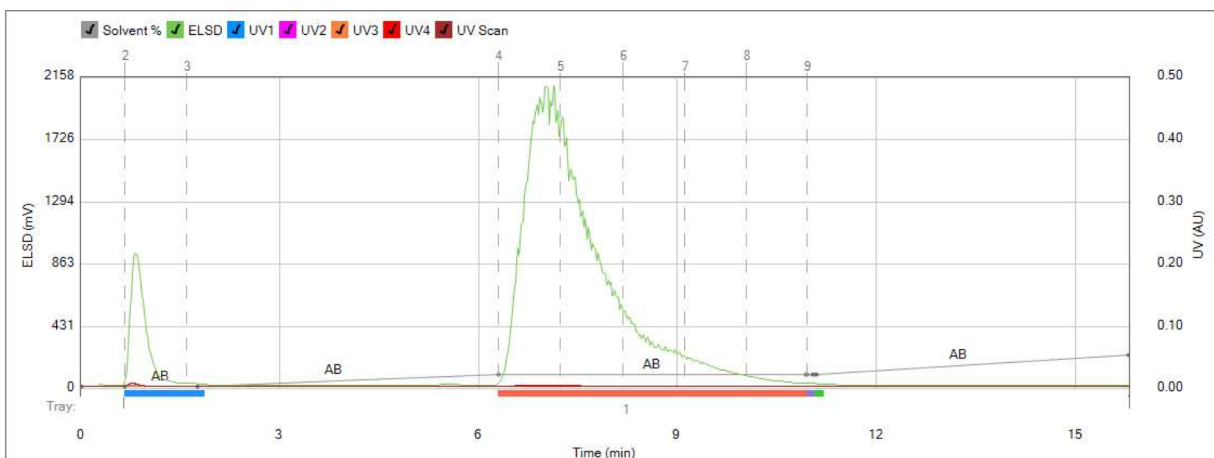
¹H NMR (400 MHz, CDCl₃) δ 5.37 – 5.32 (m, 2H), 4.66 – 4.41 (m, 1H), 3.64 – 2.95 (m, 7H), 2.43 (d, J = 125.1 Hz, 9H), 2.00 (q, J = 6.2 Hz, 7H), 1.83 – 1.54 (m, 9H), 1.54 – 0.93 (m, 49H), 0.87 (td, J = 7.0, 3.4 Hz, 9H).



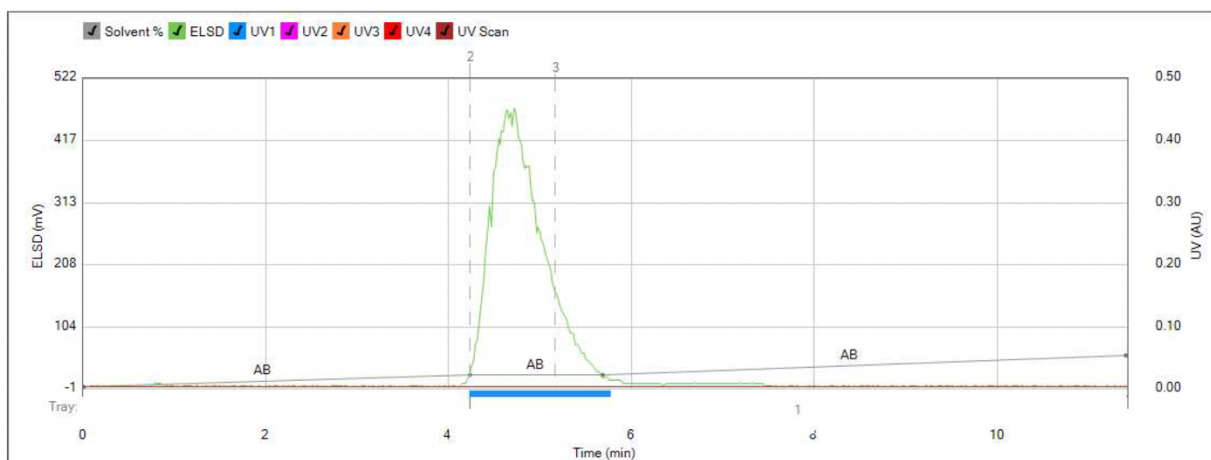
Supplementary Fig. 3 | NMR Spectrum for SC-Lipid-1.



Supplementary Fig. 4 | NMR Spectrum for SC-Lipid-1 (δ 0.5–5.5 ppm region).

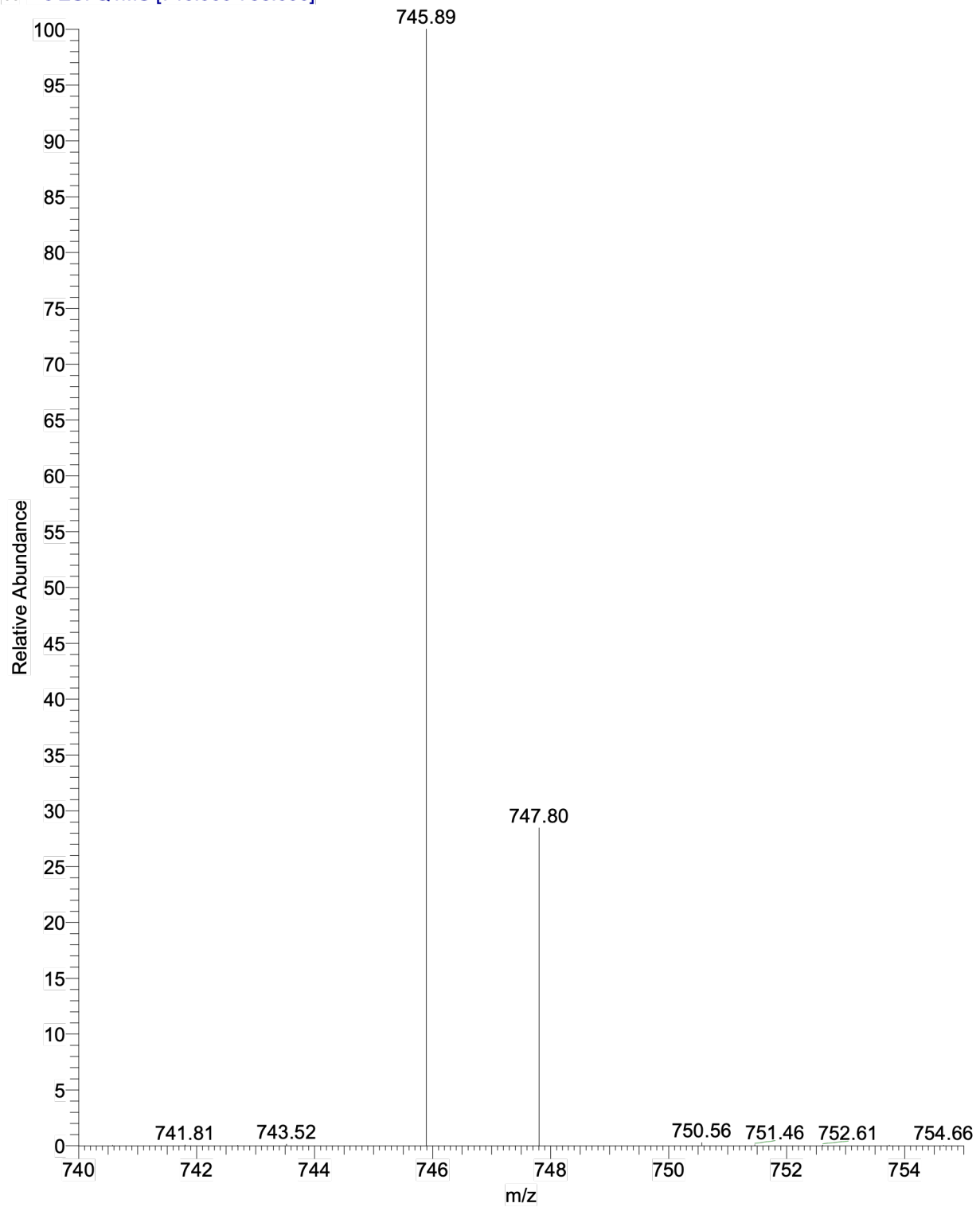


Supplementary Fig. 5 | Purification analysis of SC-lipid-1 (ELSD-HPLC).



Supplementary Fig. 6 | Purity analysis of SC-lipid-1 (ELSD-HPLC).

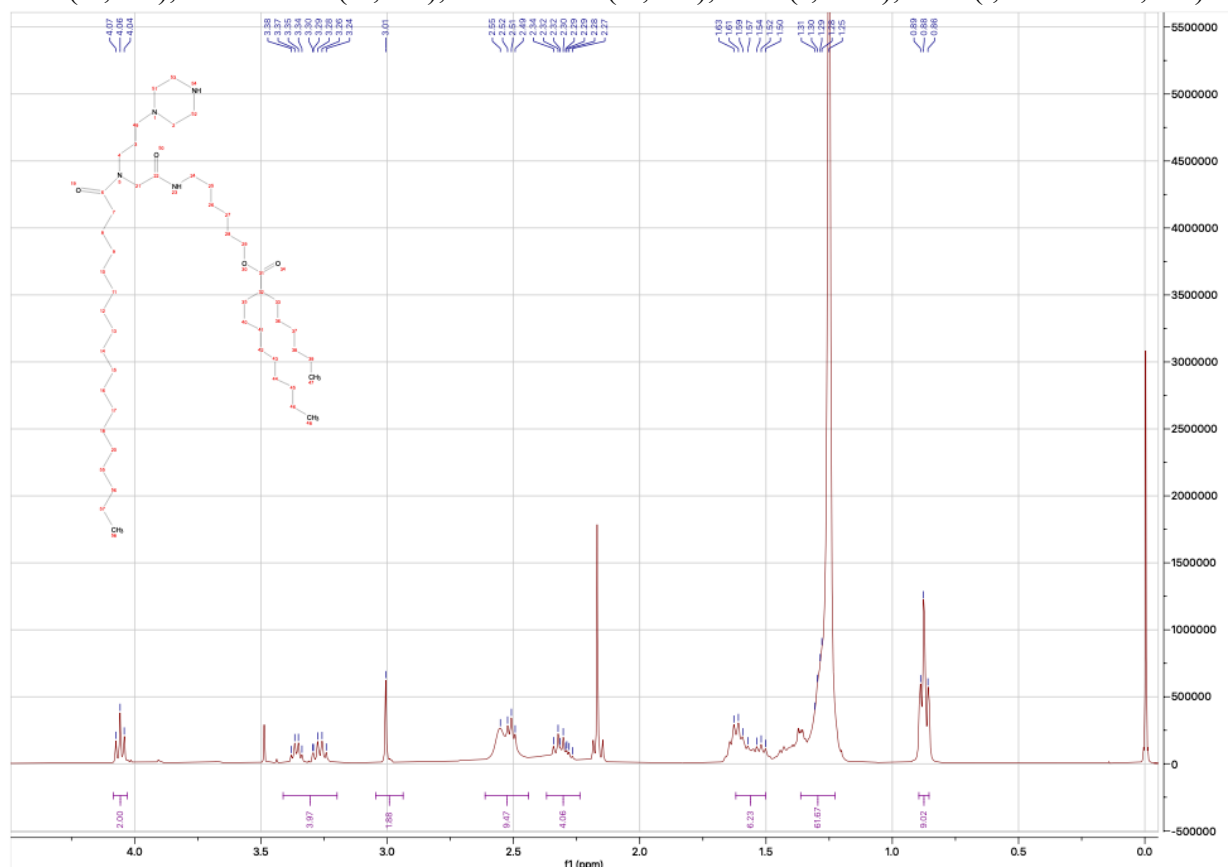
Rawdata_20260228041213 #1 RT: 0.00 AV: 1 NL: 5.53E8
T: + c ESI Q1MS [740.000-755.000]



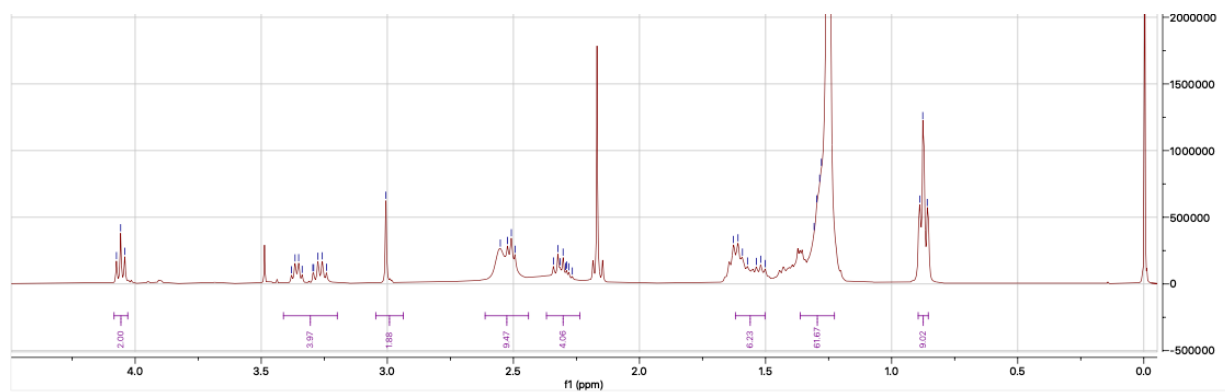
Supplementary Fig. 7 | Mass spectrometry spectrum of SC-lipid-1.

SC-Lipid-2(C₅₀H₉₈N₄O₄; MW: 819.36):

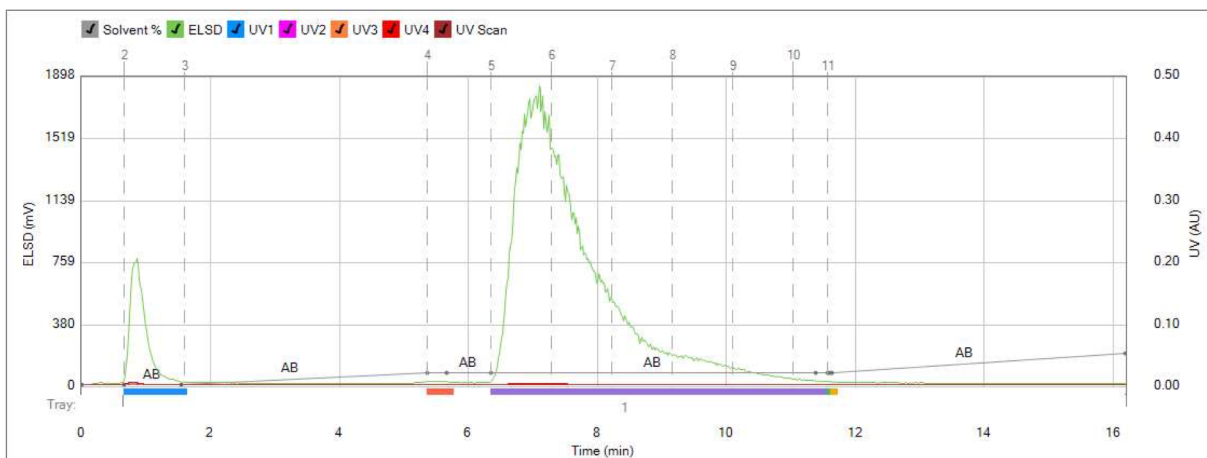
¹H NMR (400 MHz, CDCl₃) δ 4.06 (t, J = 6.6 Hz, 2H), 3.41 – 3.20 (m, 4H), 3.01 (s, 2H), 2.61 – 2.44 (m, 9H), 2.37 – 2.24 (m, 4H), 1.62 – 1.50 (m, 6H), 1.25 (s, 62H), 0.87 (t, J = 5.8 Hz, 9H).



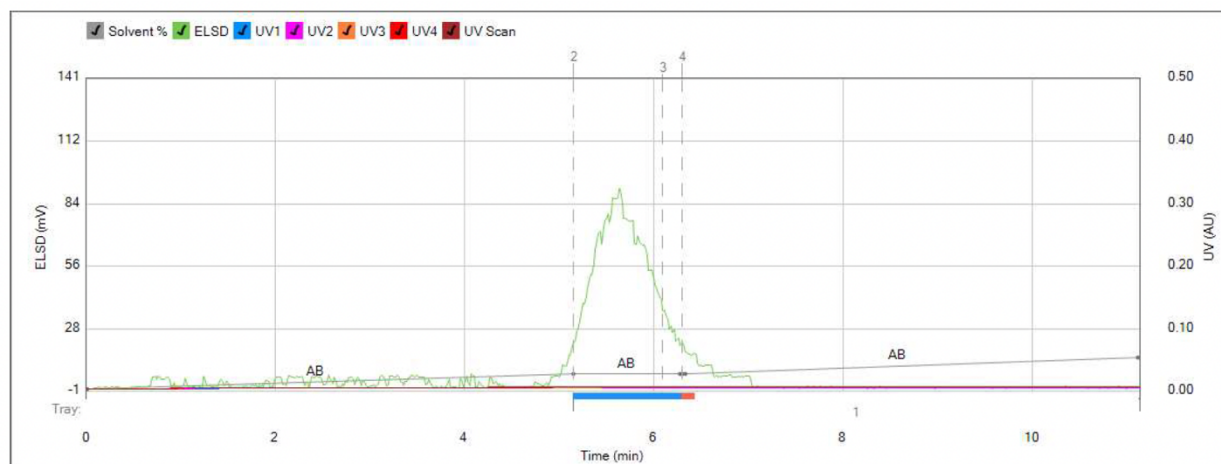
Supplementary Fig. 8 | NMR Spectrum for SC-Lipid-2.



Supplementary Fig. 9 | NMR Spectrum for SC-Lipid-2 (δ 0.5-4.2 ppm region).

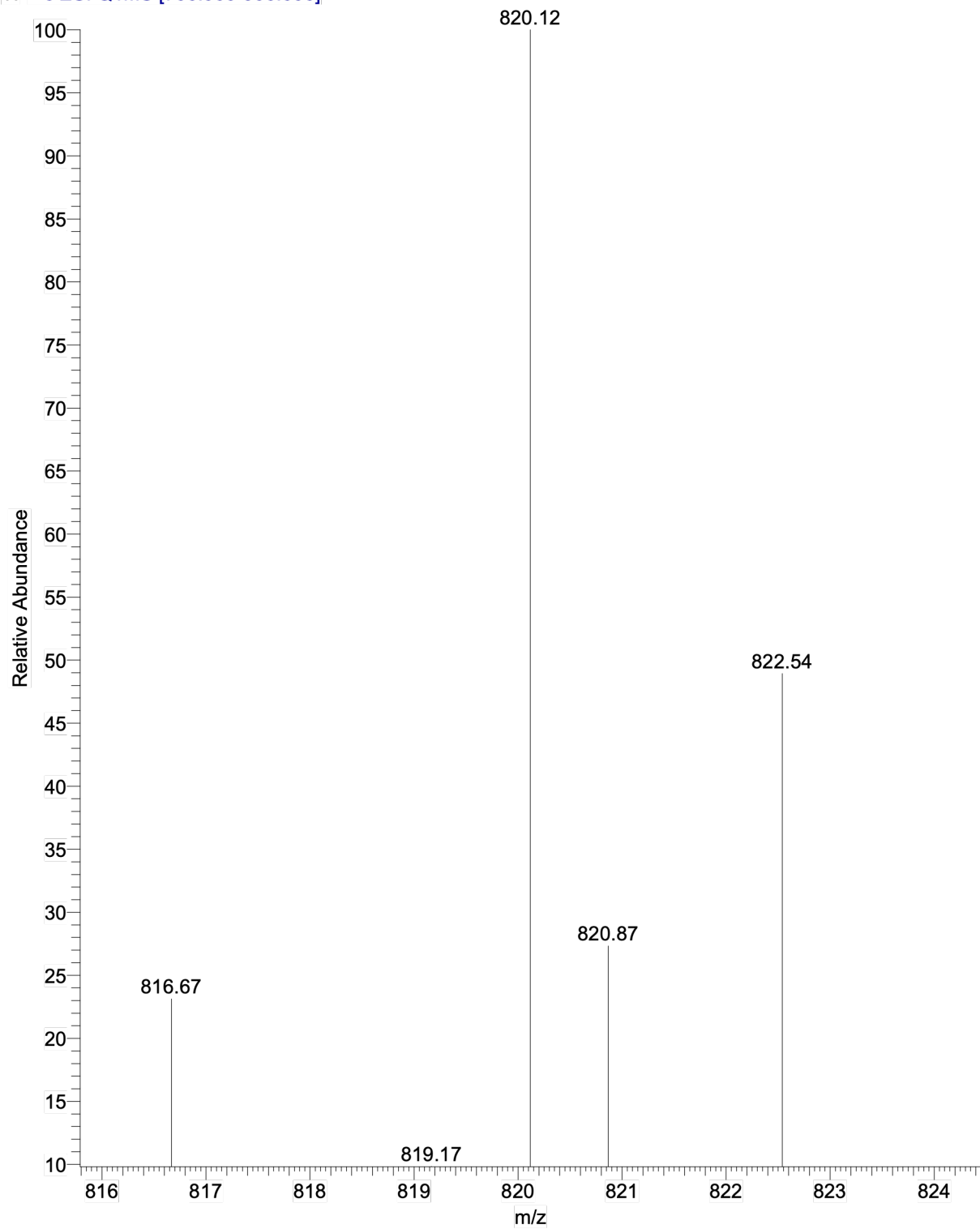


Supplementary Fig. 10 | Purification analysis of SC-lipid-2 (ELSD-HPLC).



Supplementary Fig. 11 | Purity analysis of SC-lipid-2 (ELSD-HPLC).

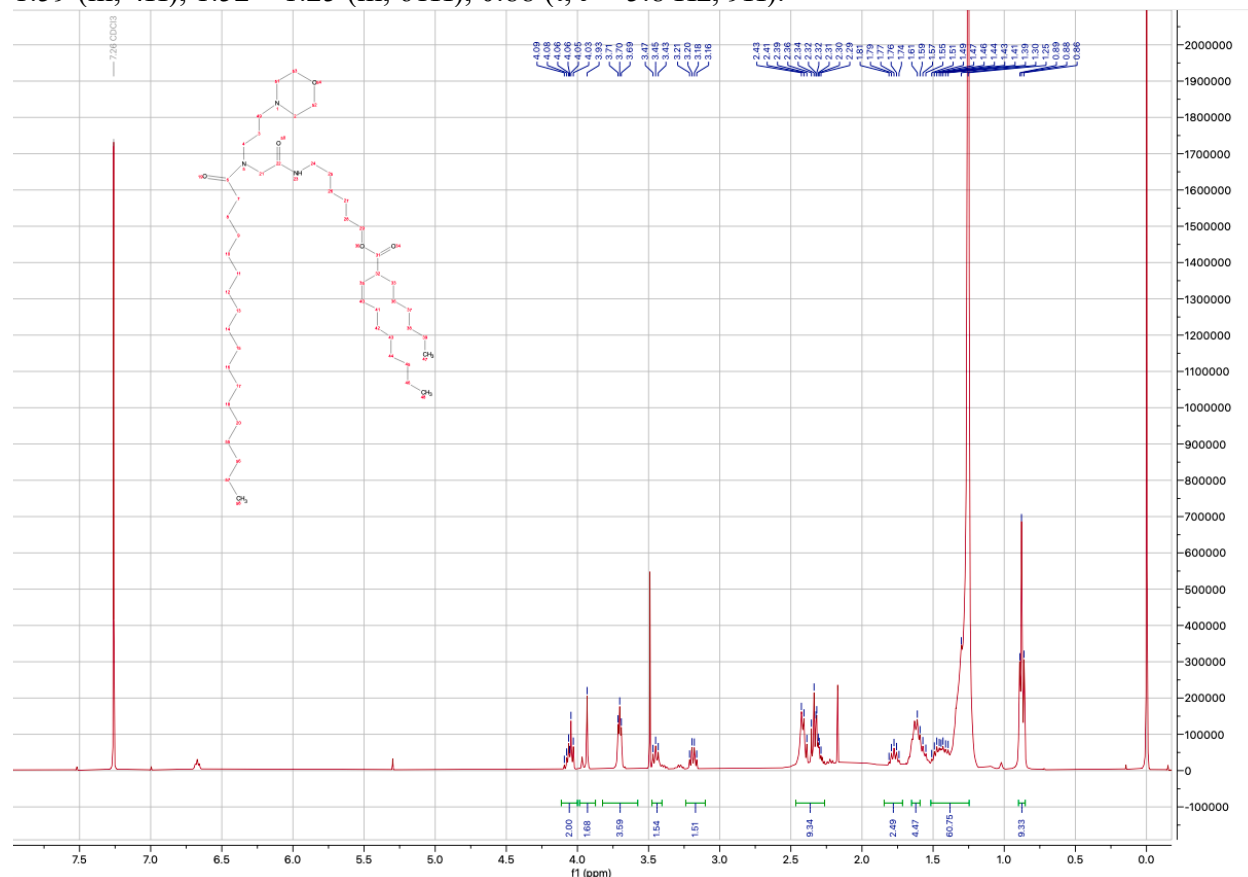
Rawdata_20260228033243 #410 RT: 1.38 AV: 1 NL: 2.07E4
T: + c ESI Q1MS [700.000-900.000]



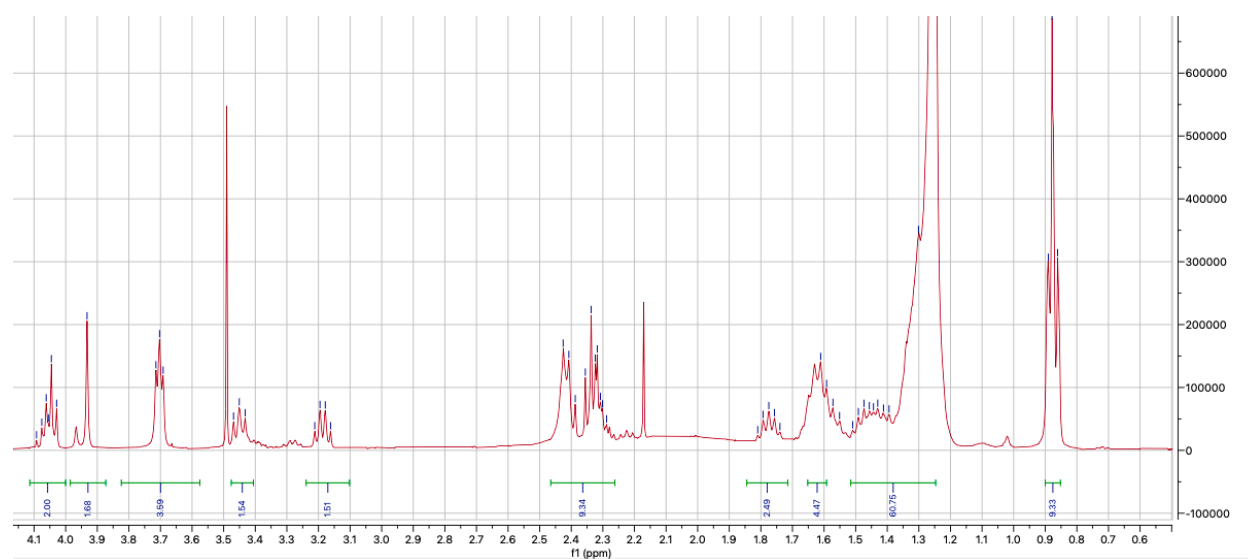
Supplementary Fig. 12 | Mass spectrometry spectrum of SC-lipid-2.

SC-Lipid-3(C₅₀H₉₇N₃O₅; MW: 820.34):

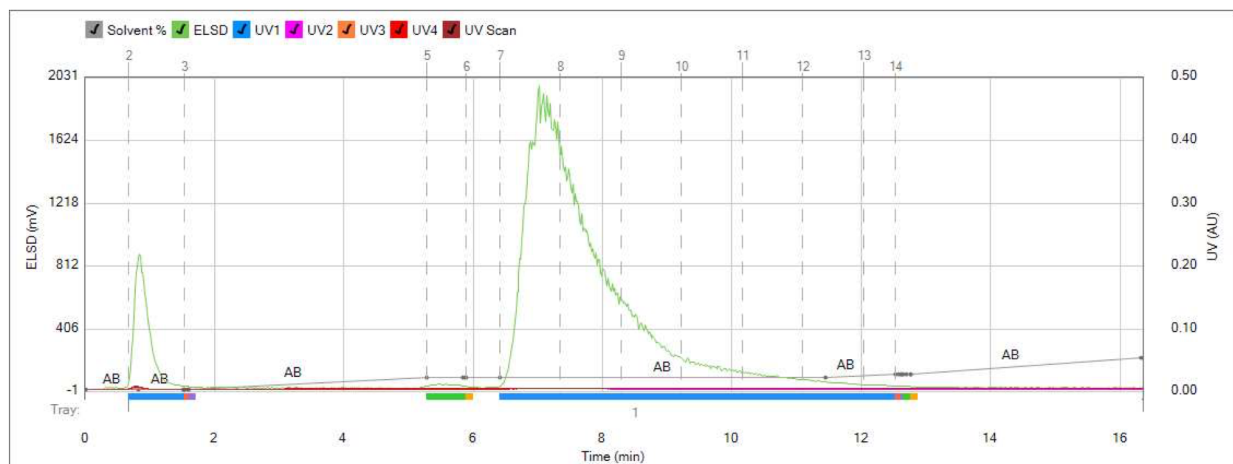
¹H NMR (400 MHz, CDCl₃) δ 4.06 (t, J = 6.7 Hz, 2H), 3.93 (s, 2H), 3.70 (t, J = 4.6 Hz, 4H), 3.45 (t, J = 7.4 Hz, 2H), 3.24 – 3.10 (m, 2H), 2.46 – 2.26 (m, 9H), 1.84 – 1.72 (m, 2H), 1.65 – 1.59 (m, 4H), 1.52 – 1.25 (m, 61H), 0.88 (t, J = 5.8 Hz, 9H).



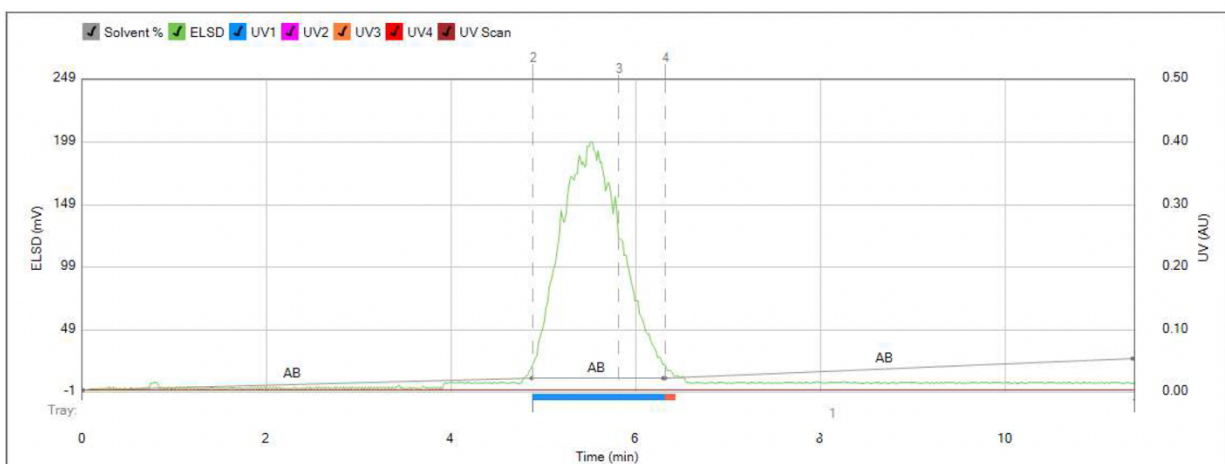
Supplementary Fig. 13 | NMR Spectrum for SC-Lipid-3.



Supplementary Fig. 14 | NMR Spectrum for SC-Lipid-3 (δ 0.5-4.2 ppm region).

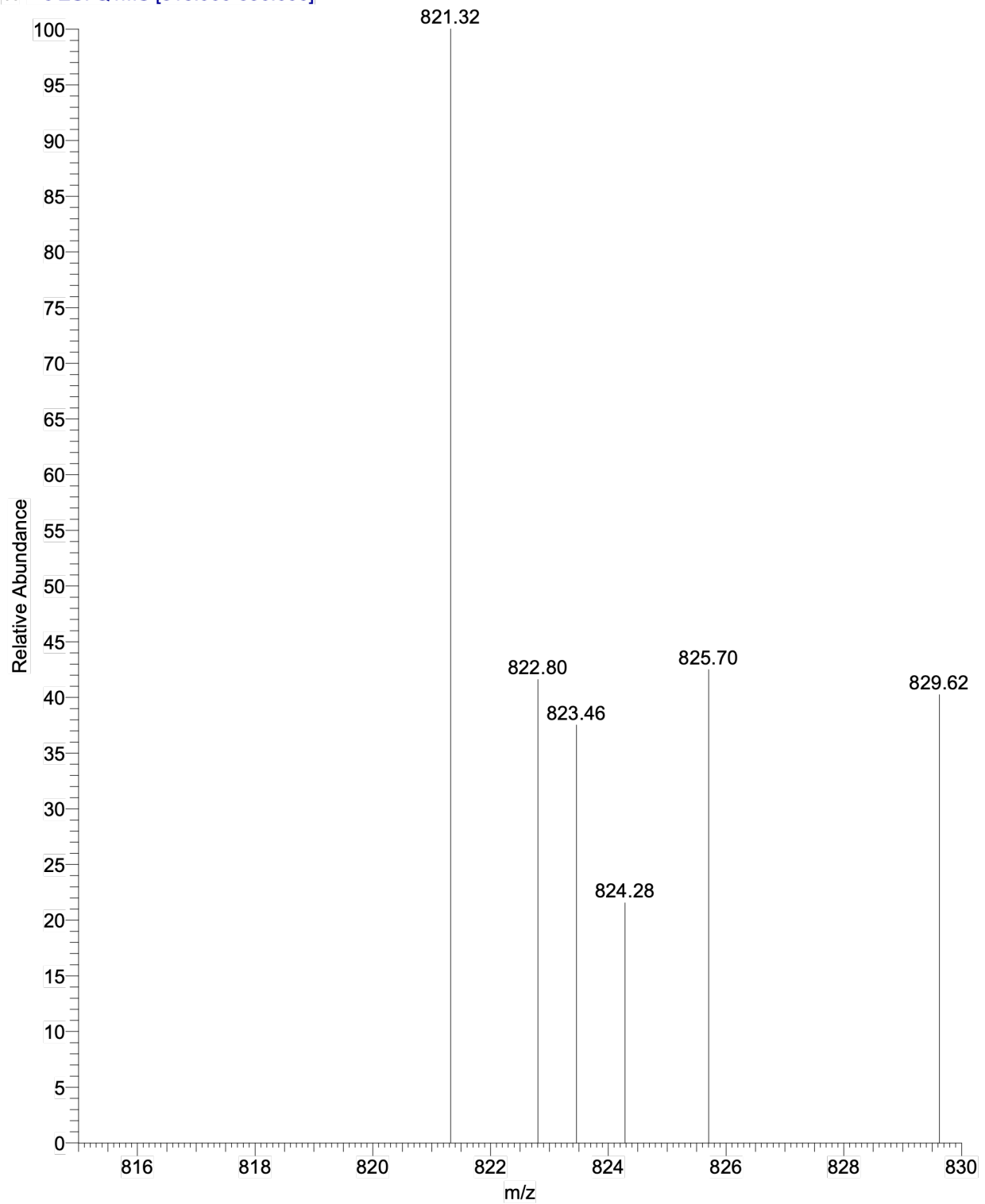


Supplementary Fig. 15 | Purification analysis of SC-lipid-3 (ELSD-HPLC).



Supplementary Fig. 16 | Purity analysis of SC-lipid-3 (ELSD-HPLC).

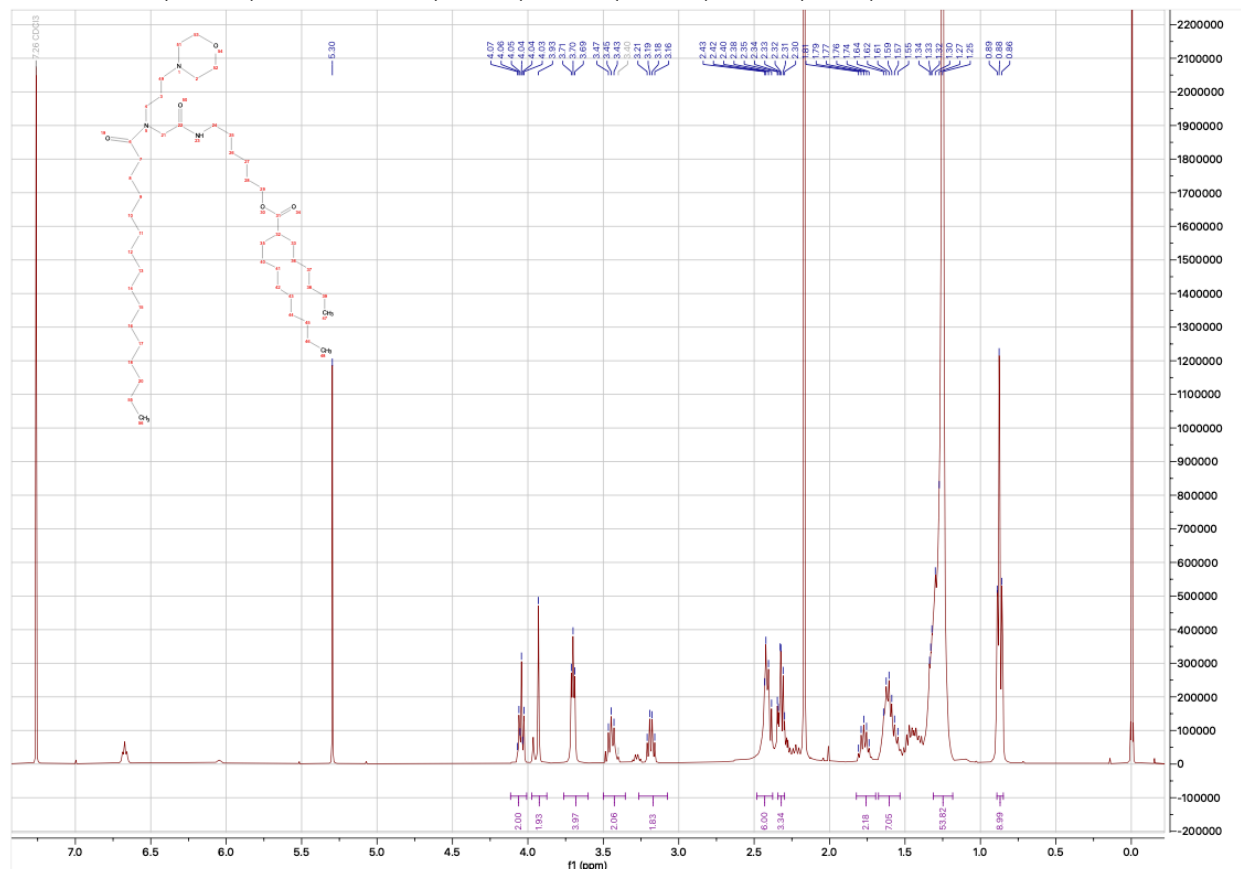
Rawdata_20260228041806 #11 RT: 0.00 AV: 1 NL: 1.93E4
T: + c ESI Q1MS [815.000-830.000]



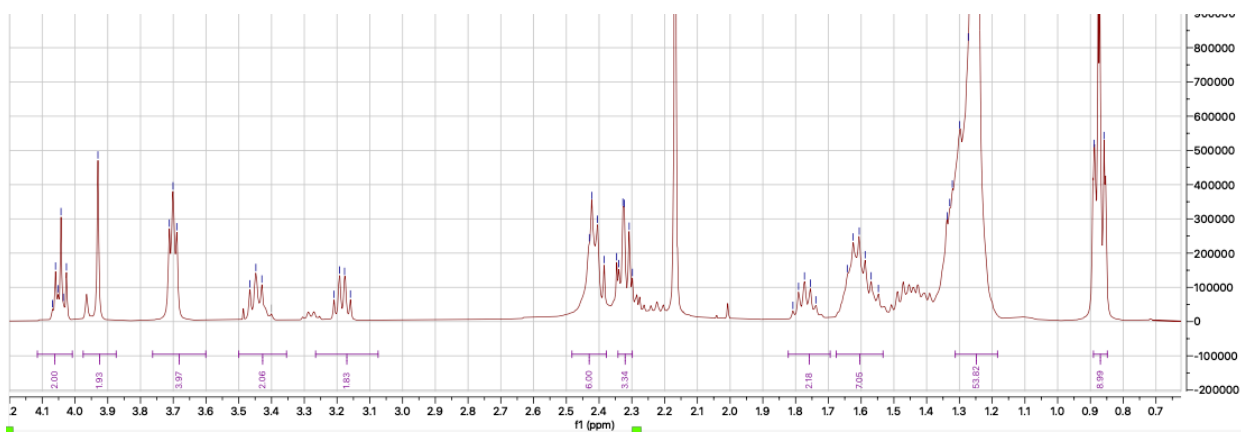
Supplementary Fig. 17 | Mass spectrometry spectrum of SC-lipid-3.

SC-Lipid-4(C₄₈H₉₃N₃O₅; MW: 792.29):

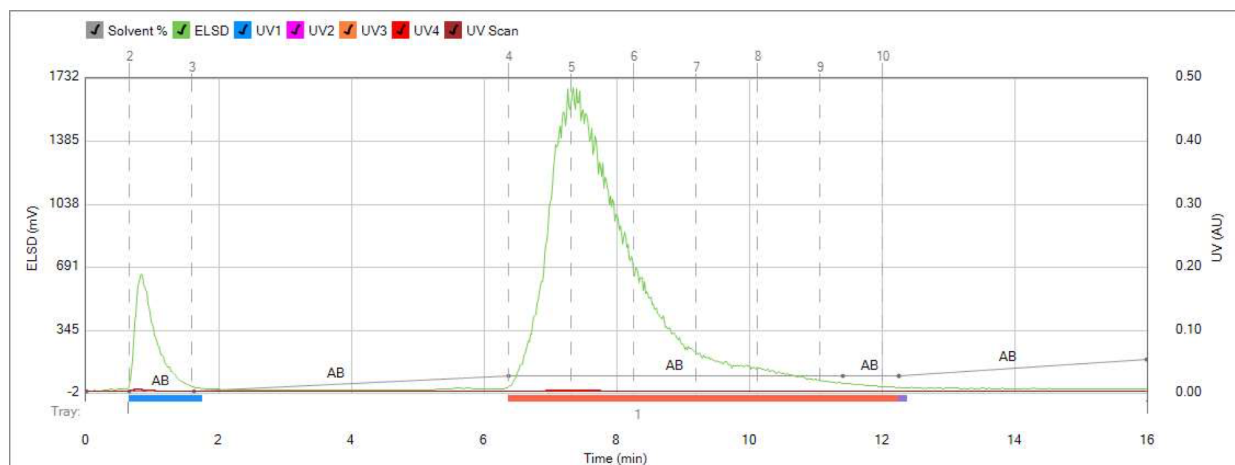
¹H NMR (400 MHz, CDCl₃) δ 4.05 (td, J = 6.7, 3.8 Hz, 2H), 3.93 (s, 2H), 3.70 (t, J = 4.6 Hz, 4H), 3.45 (t, J = 7.4 Hz, 2H), 3.18 (q, J = 7.0 Hz, 2H), 2.48 – 2.38 (m, 6H), 2.34 – 2.30 (m, 3H), 1.82 – 1.69 (m, 2H), 1.68 – 1.53 (m, 7H), 1.25 (s, 54H), 0.87 (t, 9H).



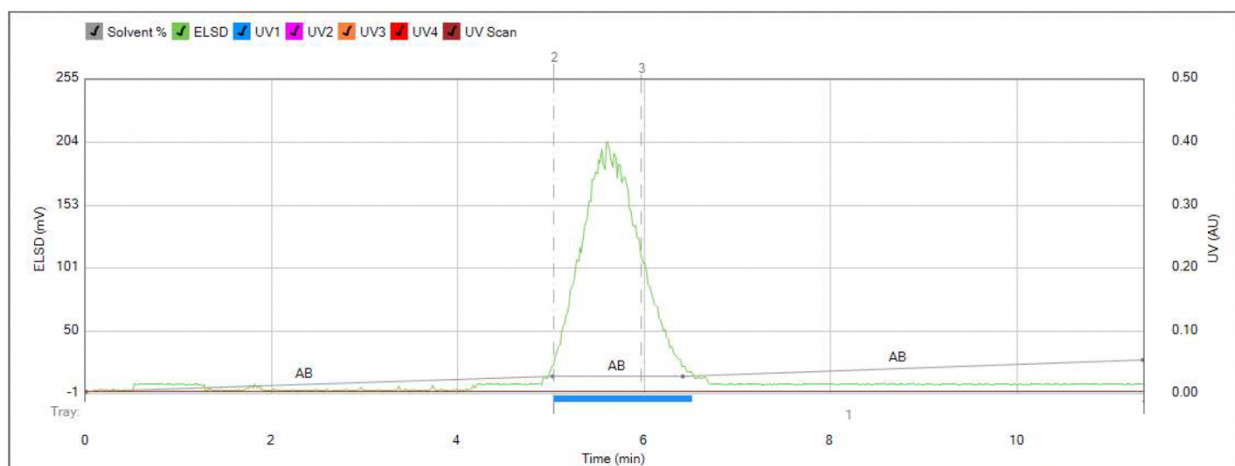
Supplementary Fig. 18 | NMR Spectrum for SC-Lipid-4.



Supplementary Fig. 19 | NMR Spectrum for SC-Lipid-4 (δ 0.5-4.2 ppm region)..

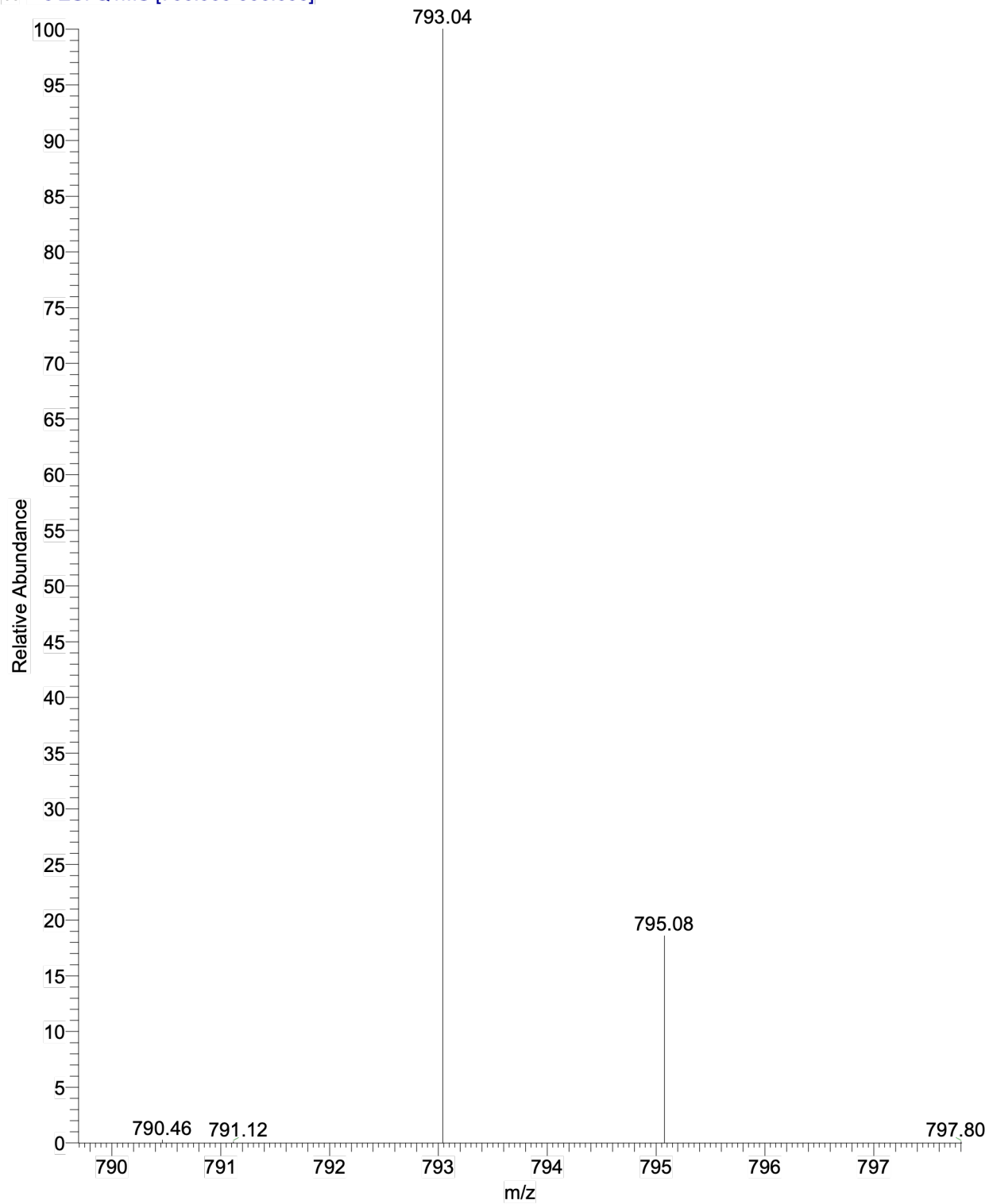


Supplementary Fig. 20 | Purification analysis of SC-lipid-4 (ELSD-HPLC).



Supplementary Fig. 21 | Purity analysis of SC-lipid-4 (ELSD-HPLC).

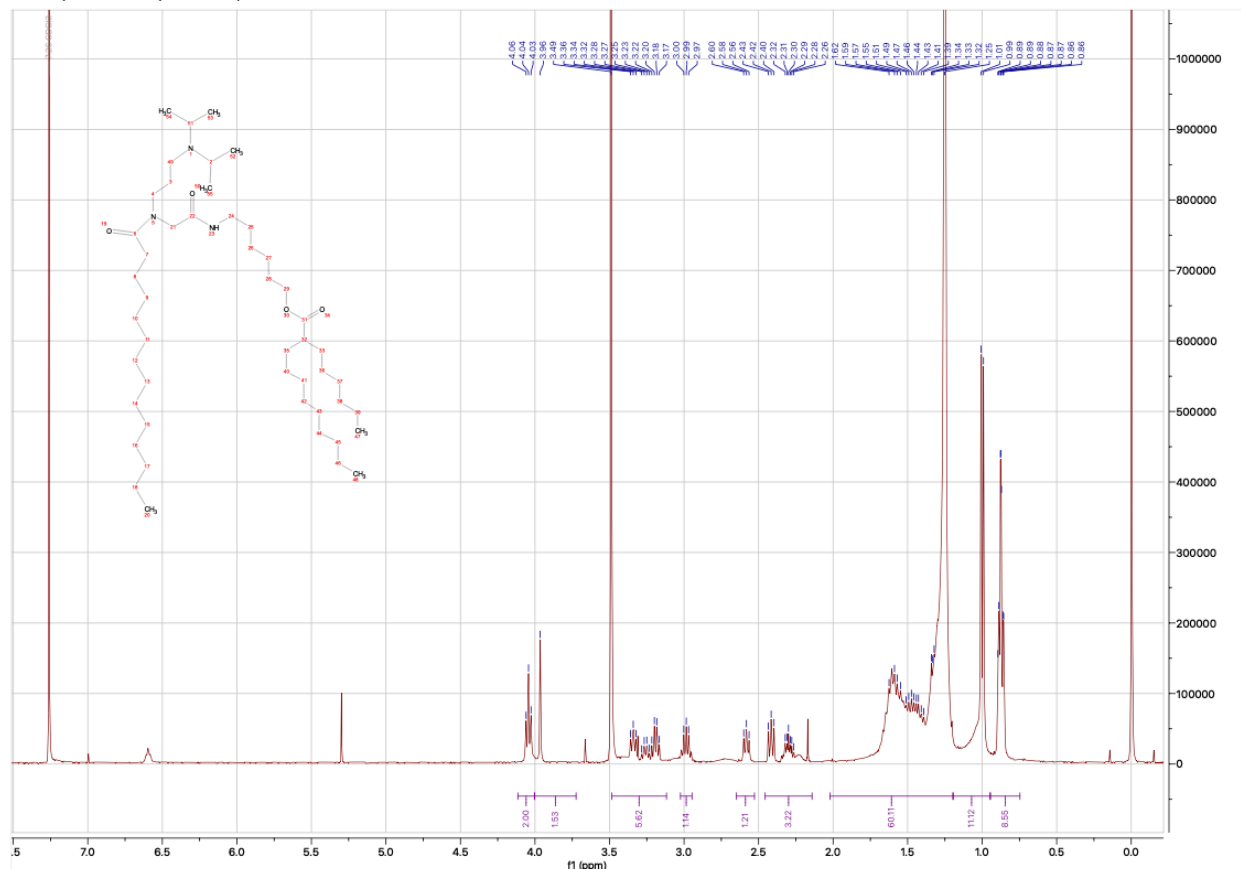
Rawdata_20260228034637 #95 RT: 0.32 AV: 1 NL: 1.42E8
T: + c ESI Q1MS [700.000-900.000]



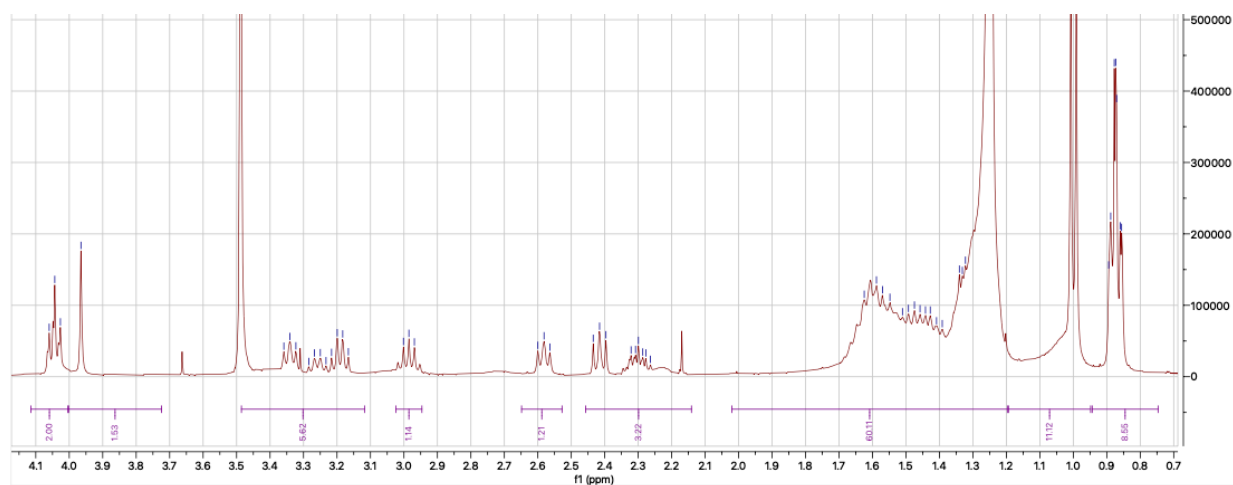
Supplementary Fig. 22 | Mass spectrometry spectrum of SC-lipid-4.

SC-Lipid-5(C₄₈H₉₅N₃O₄; MW: 778.31):

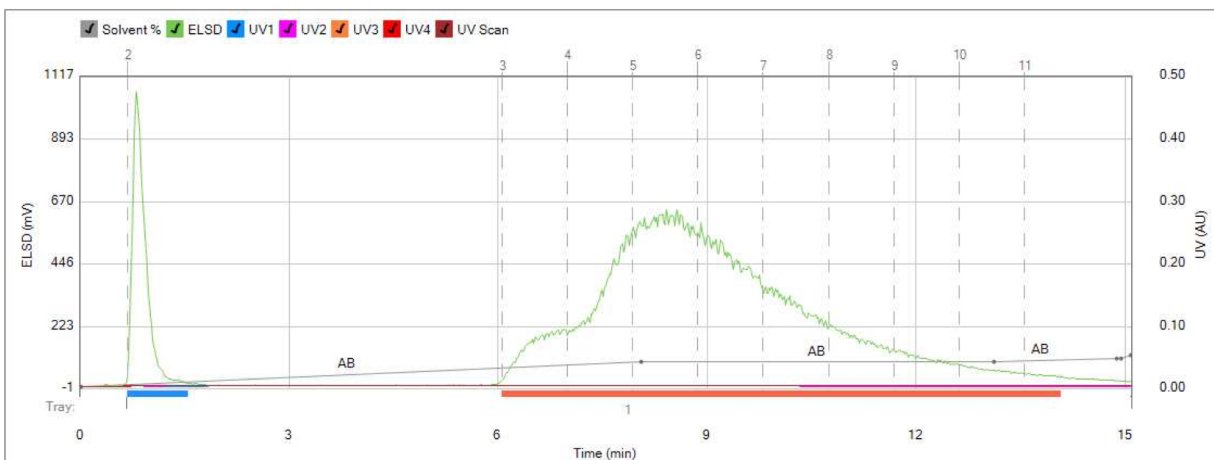
¹H NMR (400 MHz, CDCl₃) δ 4.04 (t, J = 6.7 Hz, 2H), 3.96 (s, 2H), 3.49 – 3.12 (m, 6H), 3.02 – 2.95 (m, 1H), 2.58 (t, J = 7.2 Hz, 1H), 2.46 – 2.14 (m, 3H), 1.25 (s, 60H), 1.00 (d, J = 6.5 Hz, 11H), 0.87 (tt, 9H).



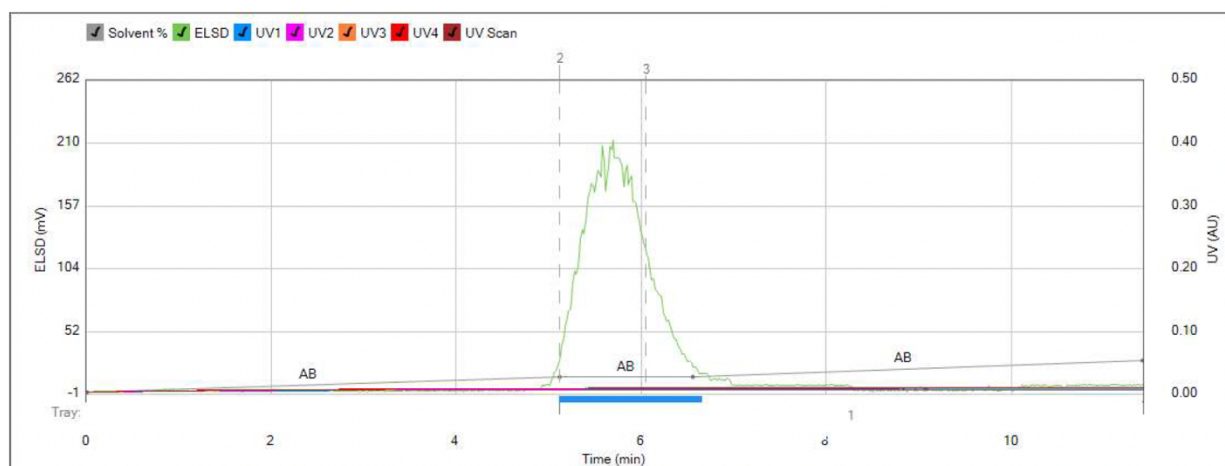
Supplementary Fig. 23 | NMR Spectrum for SC-Lipid-5.



Supplementary Fig. 24 | NMR Spectrum for SC-Lipid-5 (δ 0.5-4.2 ppm region)..

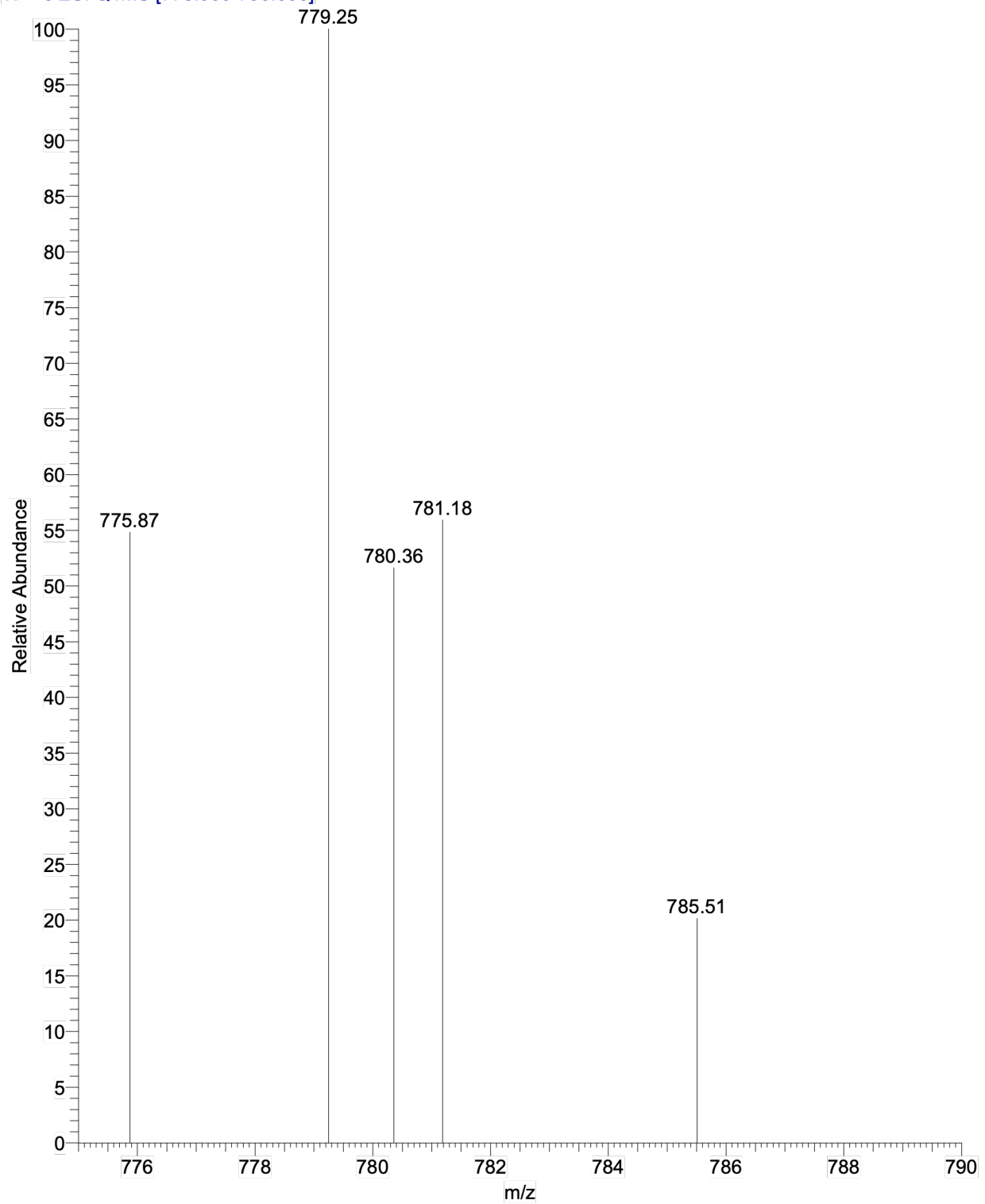


Supplementary Fig. 25 | Purification analysis of SC-lipid-5 (ELSD-HPLC).



Supplementary Fig. 26 | Purity analysis of SC-lipid-5 (ELSD-HPLC).

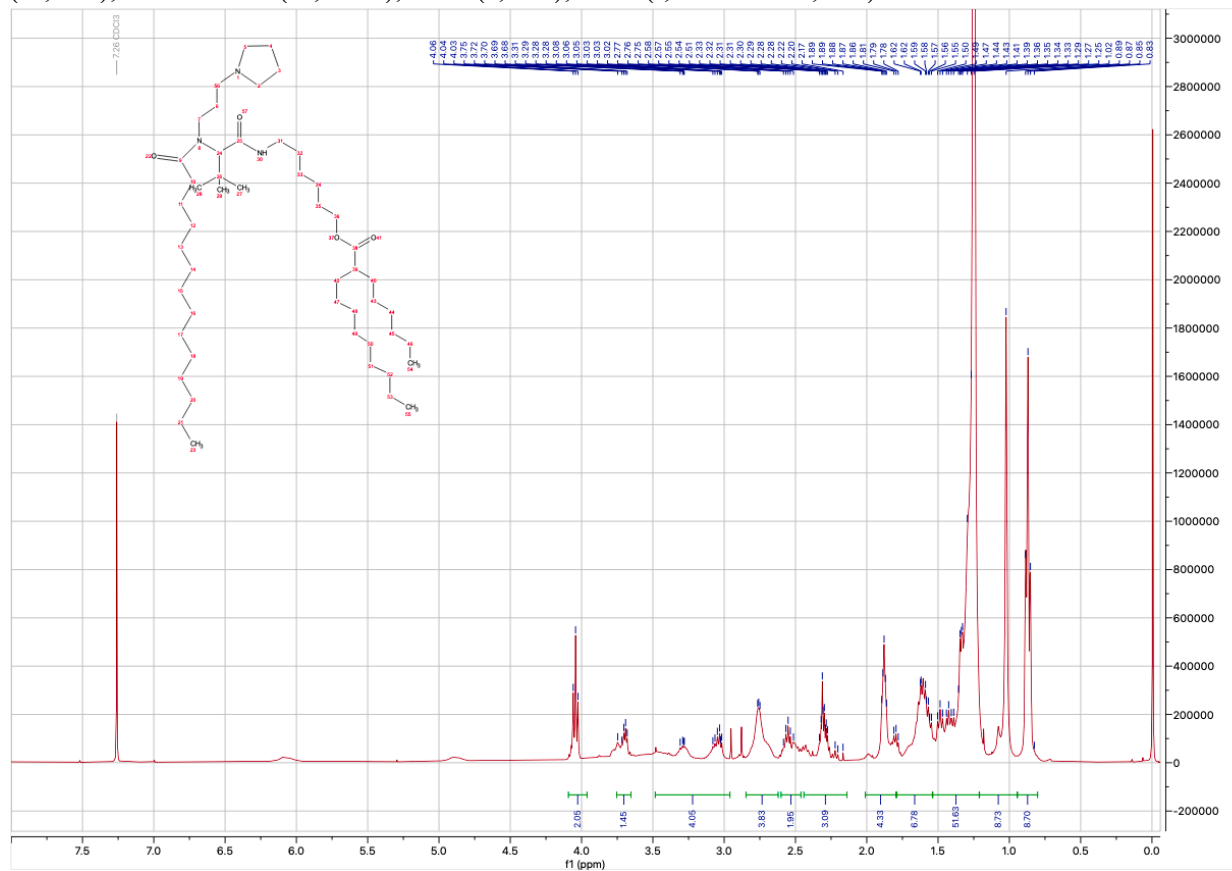
Rawdata_20260228042442 #4 RT: 0.00 AV: 1 NL: 1.19E4
T: + c ESI Q1MS [775.000-790.000]



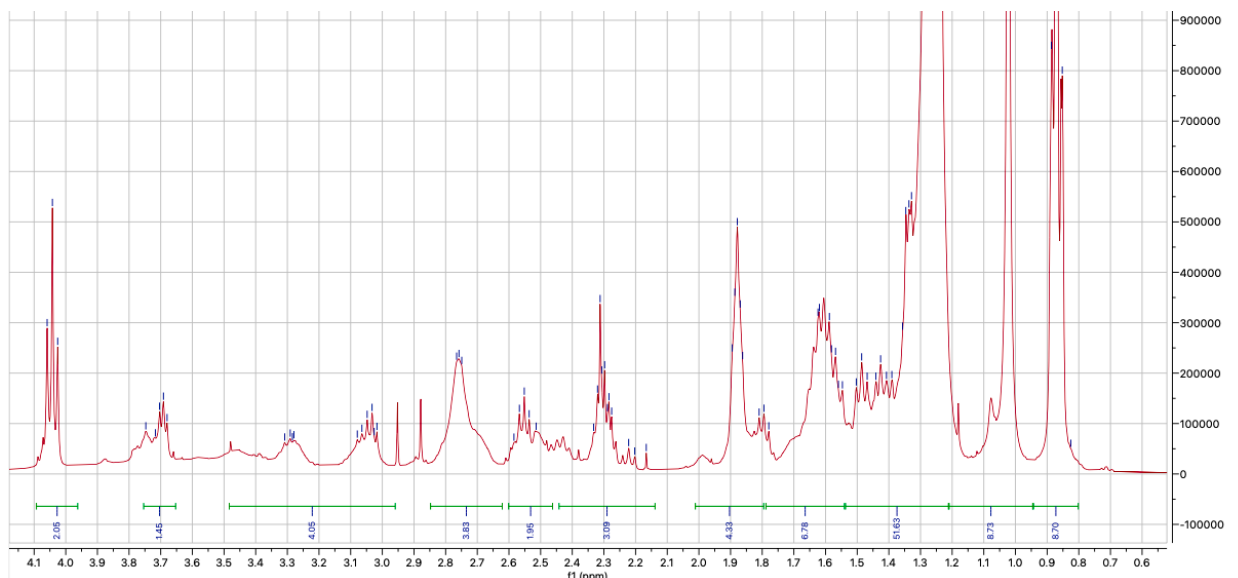
Supplementary Fig. 27 | Mass spectrometry spectrum of SC-lipid-5.

LC-Lipid-1(C₅₀H₉₇N₃O₄; MW: 804.34):

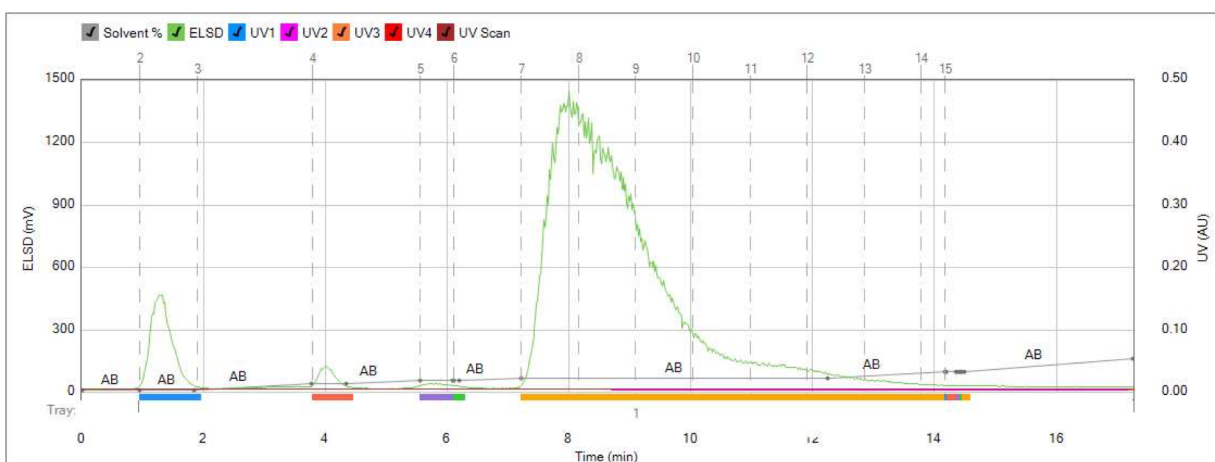
¹H NMR (400 MHz, CDCl₃) δ 4.04 (t, J = 6.7 Hz, 2H), 3.75 – 3.65 (m, 1H), 3.48 – 2.96 (m, 4H), 2.85 – 2.62 (m, 4H), 2.60 – 2.46 (m, 2H), 2.44 – 2.14 (m, 3H), 2.01 – 1.80 (m, 4H), 1.79 – 1.54 (m, 7H), 1.54 – 1.21 (m, 52H), 1.02 (s, 9H), 0.87 (t, J = 6.9 Hz, 9H).



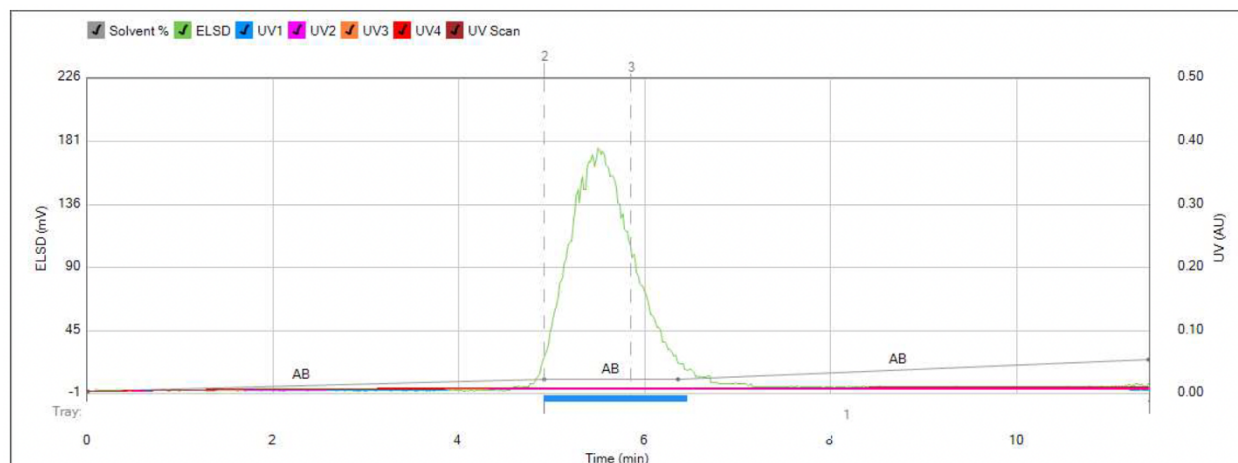
Supplementary Fig. 28 | NMR Spectrum for LC-Lipid-1.



Supplementary Fig. 29 | NMR Spectrum for LC-Lipid-1. (δ 0.5-4.2 ppm region).



Supplementary Fig. 30 | Purification analysis of LC-lipid-1 (ELSD-HPLC).



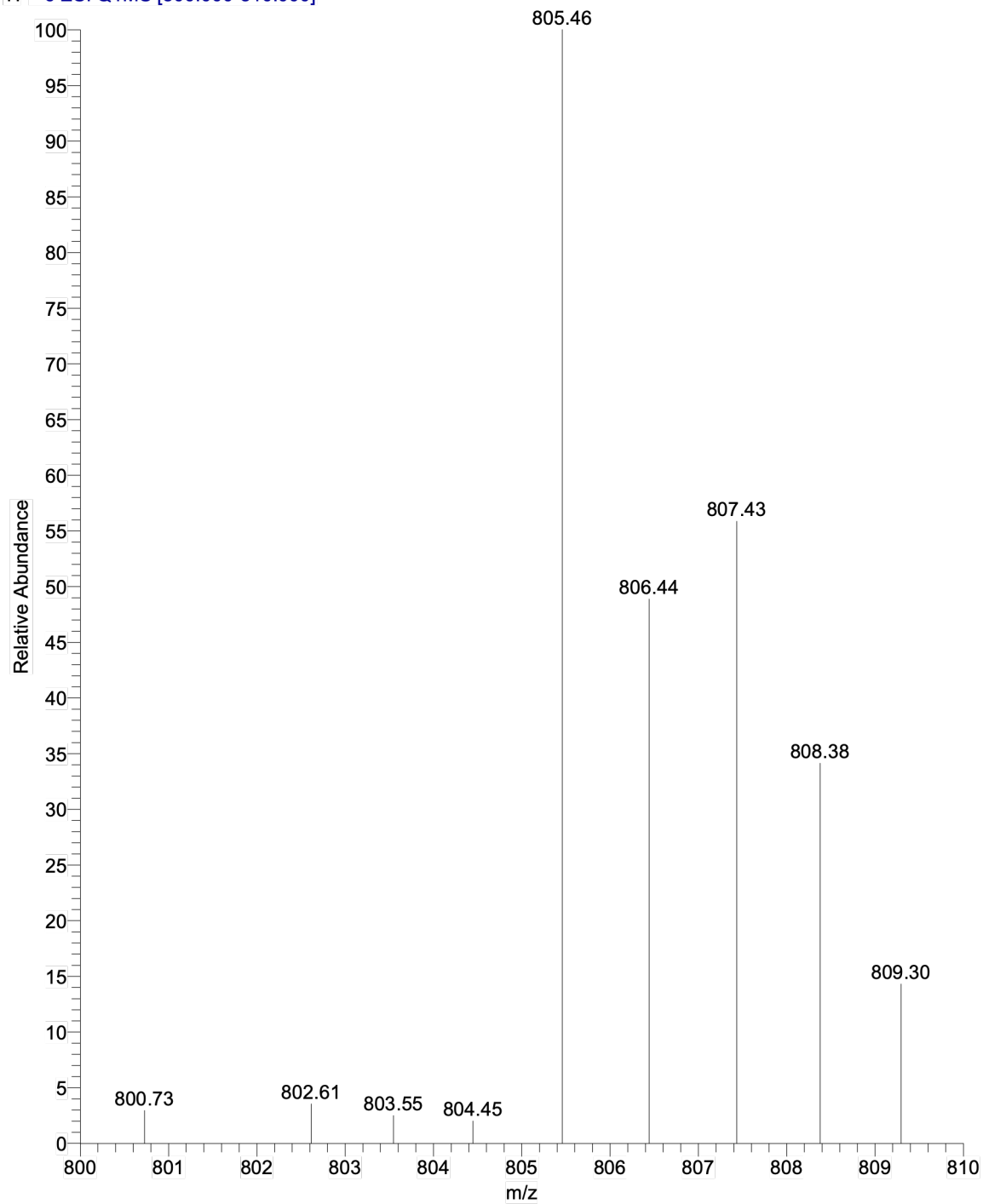
Supplementary Fig. 31 | Purity analysis of LC-lipid-1 (ELSD-HPLC).

C:\Thermo\Data\Rawdata_20260228035111

02/28/26 15:51:11

Rawdata_20260228035111 #237 RT: 0.71 AV: 1 NL: 8.84E5

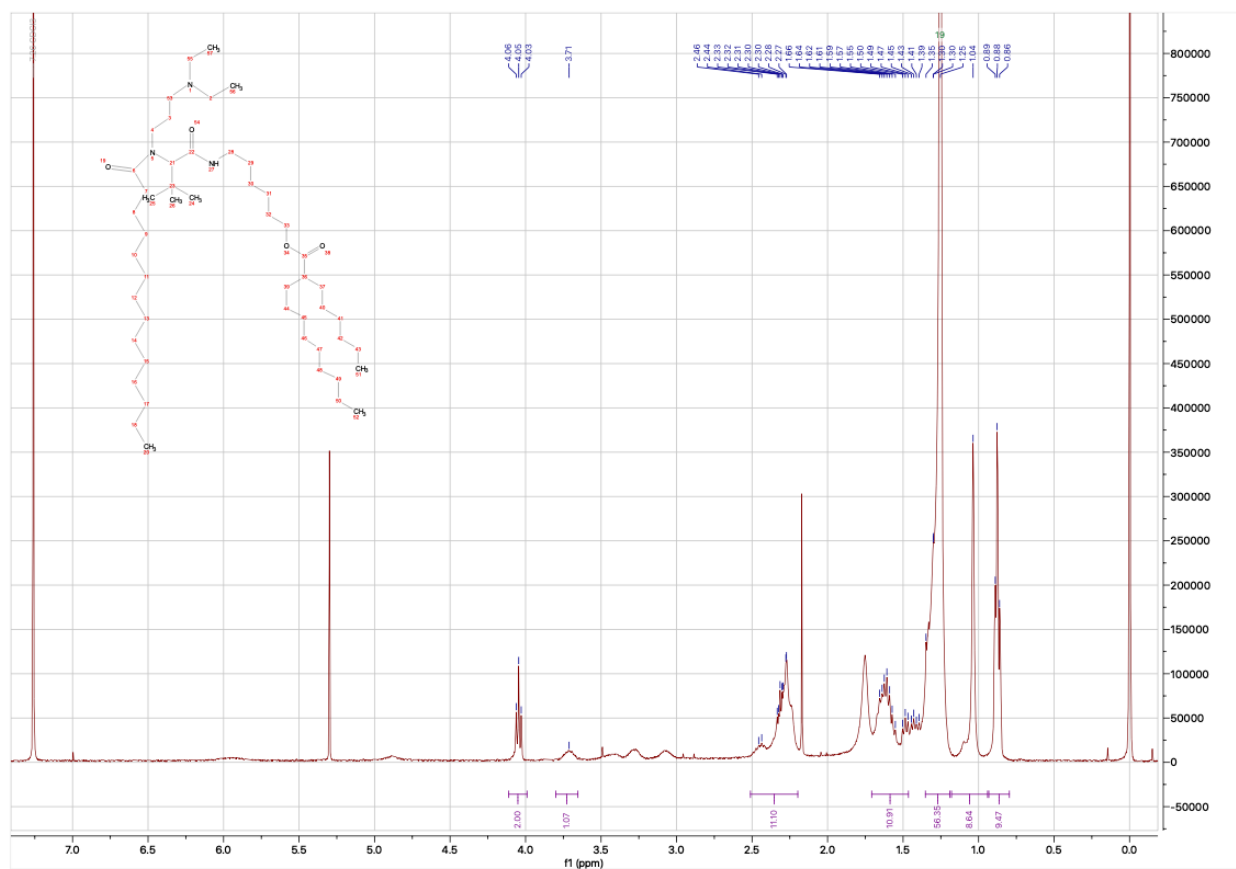
T: + c ESI Q1MS [800.000-810.000]



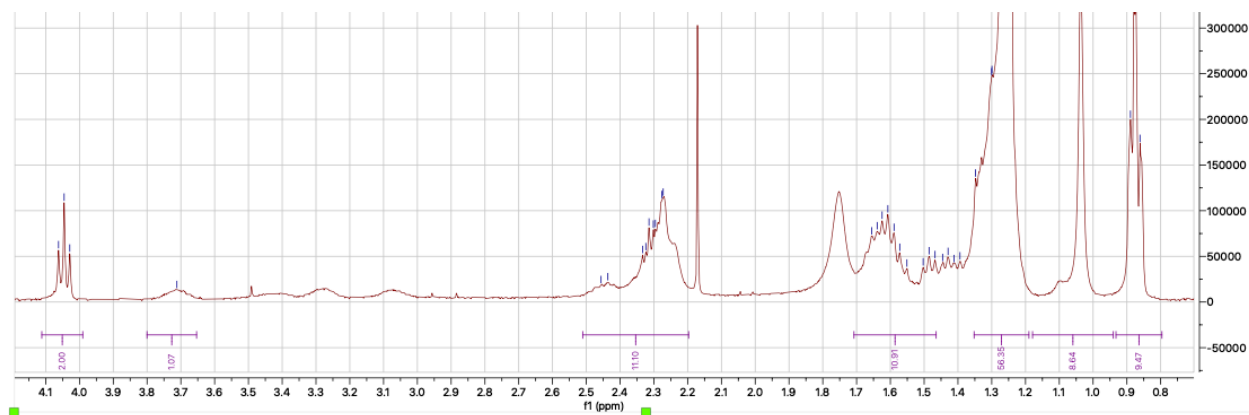
Supplementary Fig. 32 | Mass spectrometry spectrum of LC-lipid-1.

LC-Lipid-2 (C₅₀H₉₉N₃O₄; MW: 806.36):

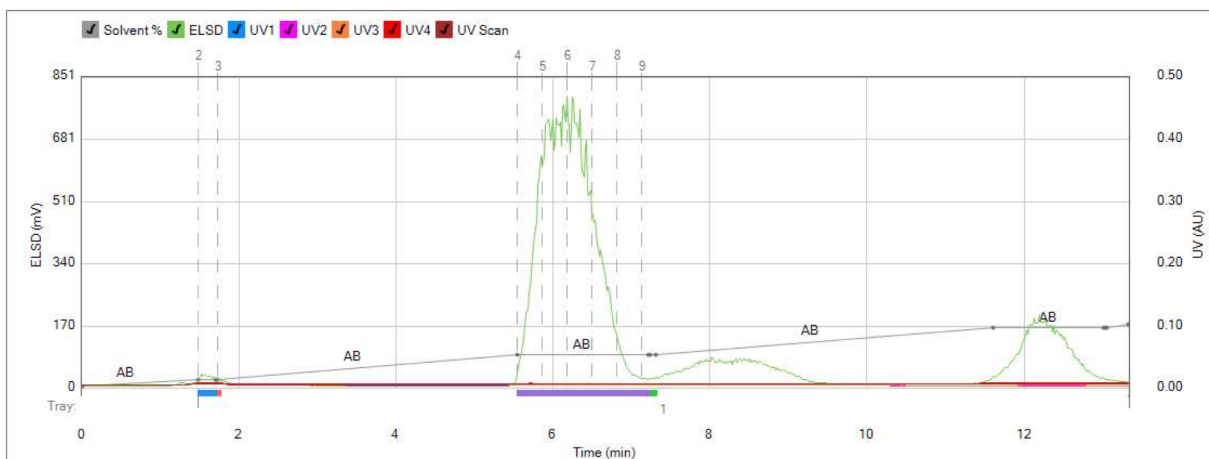
¹H NMR (400 MHz, CDCl₃) δ 4.05 (t, J = 6.7 Hz, 2H), 3.71 (s, 1H), 2.51 – 2.20 (m, 11H), 1.57 (dt, J = 62.9, 14.1, 6.8 Hz, 11H), 1.25 (s, 56H), 1.04 (s, 9H), 0.88 (d, J = 7.0 Hz, 9H).



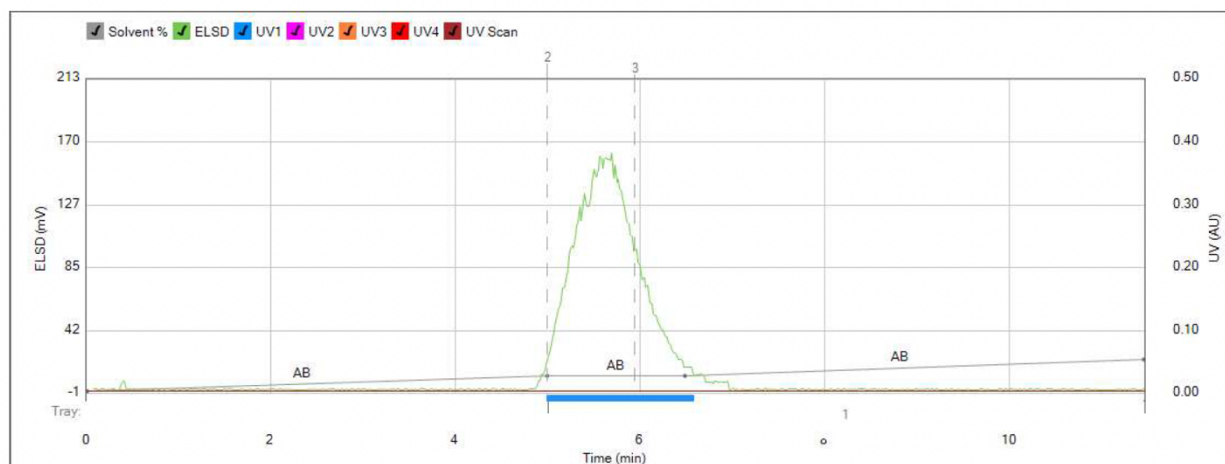
Supplementary Fig. 33 | NMR Spectrum for LC-Lipid-2.



Supplementary Fig. 34 | NMR Spectrum for LC-Lipid-2. (δ 0.5–4.2 ppm region).

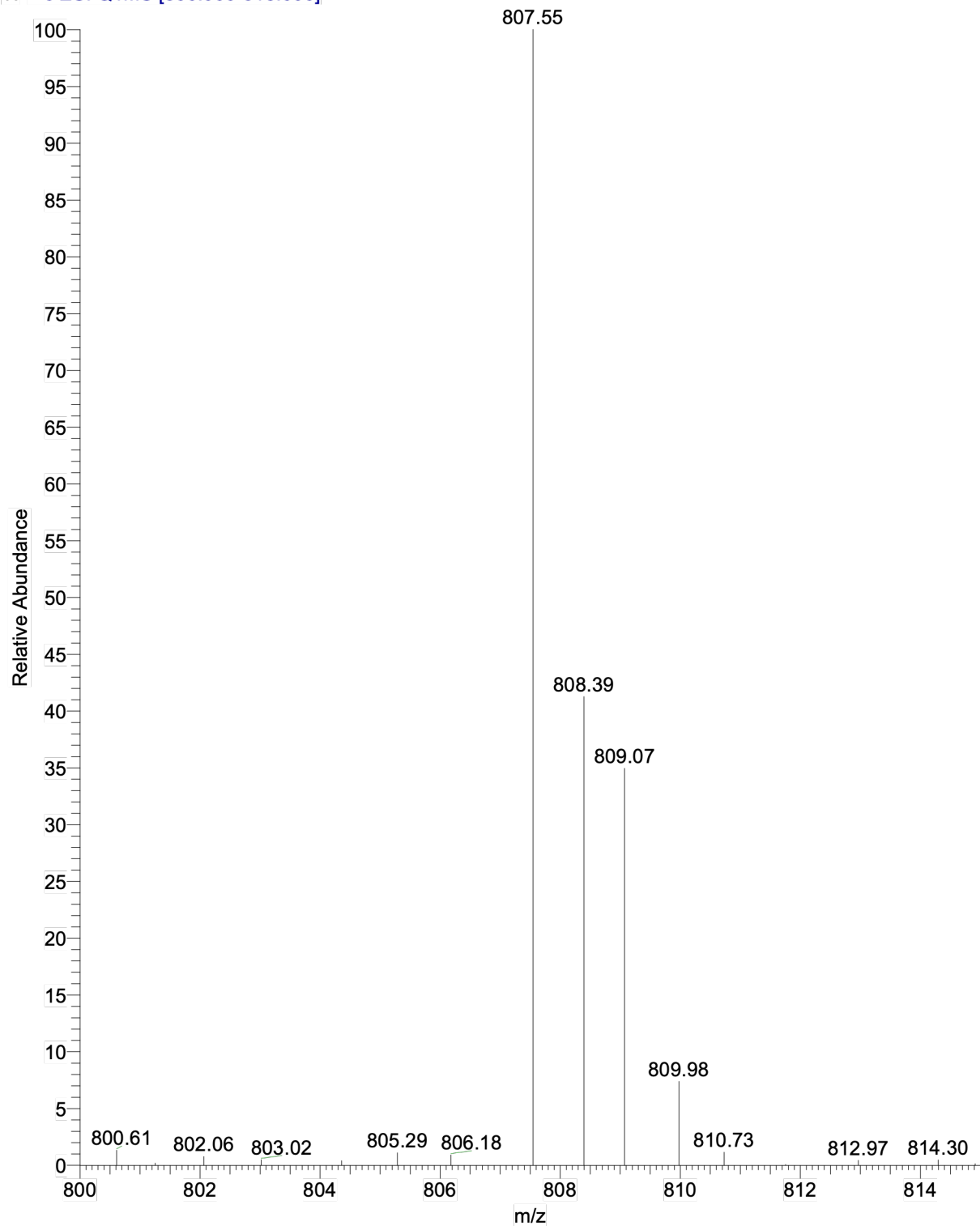


Supplementary Fig. 35 | Purification analysis of LC-lipid-2 (ELSD-HPLC).



Supplementary Fig. 36 | Purity analysis of LC-lipid-2 (ELSD-HPLC).

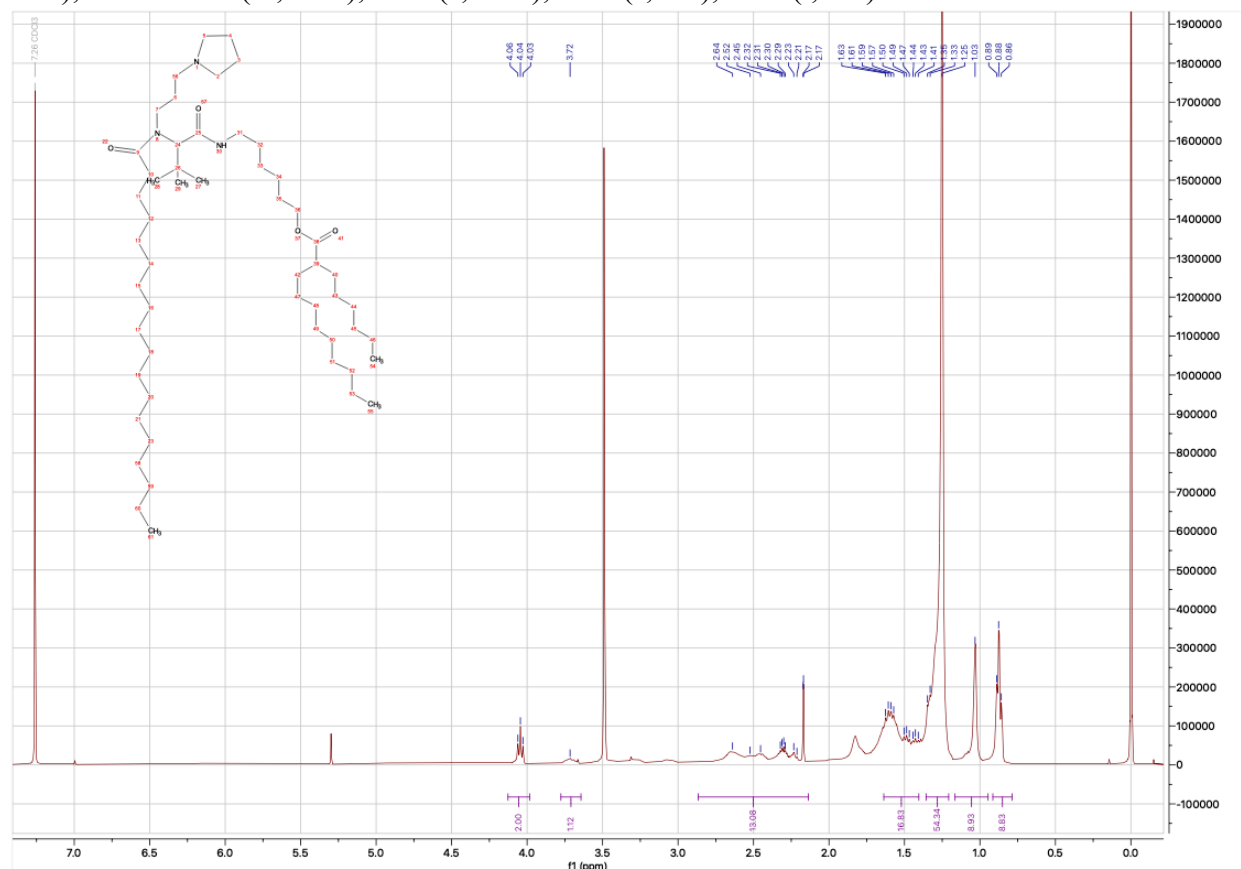
Rawdata_20260228042825 #828 RT: 0.24 AV: 1 NL: 2.80E6
T: + c ESI Q1MS [800.000-815.000]



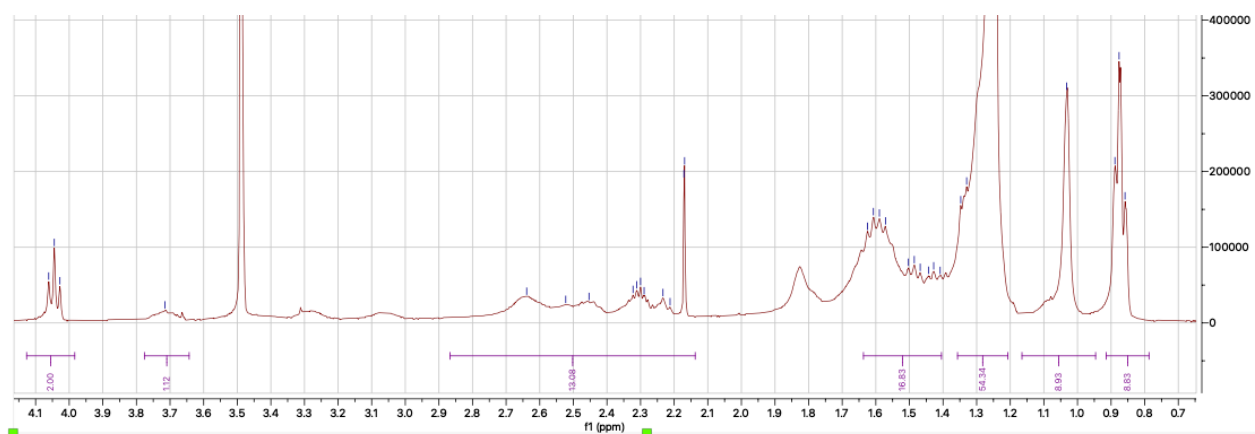
Supplementary Fig. 37 | Mass spectrometry spectrum of LC-lipid-2.

LC-Lipid-3(C₅₄H₁₀₅N₃O₄; MW: 860.45):

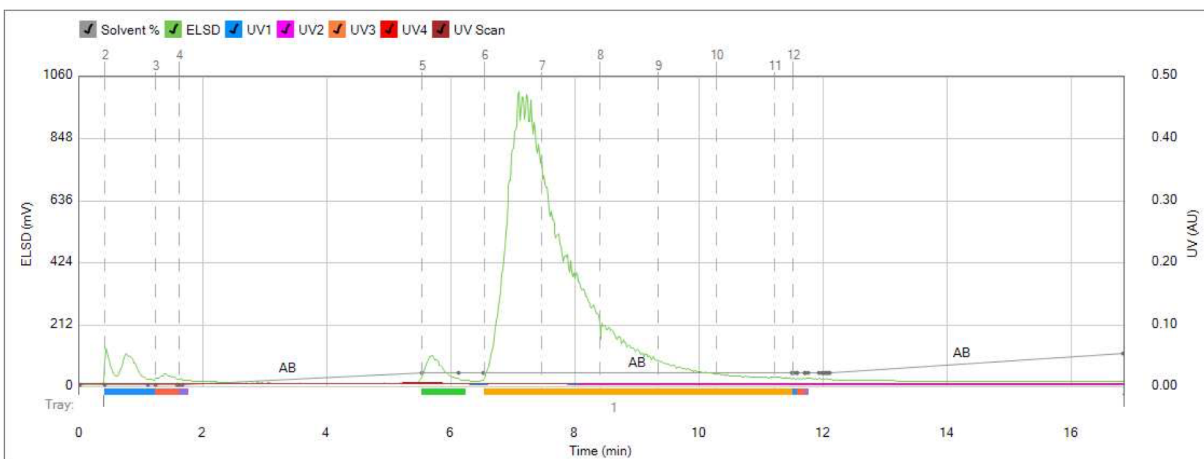
¹H NMR (400 MHz, CDCl₃) δ 4.04 (t, J = 6.7 Hz, 2H), 3.69 (d, J = 20.5 Hz, 1H), 2.87 – 2.14 (m, 13H), 1.64 – 1.40 (m, 17H), 1.25 (s, 54H), 1.03 (s, 9H), 0.87 (t, 9H).



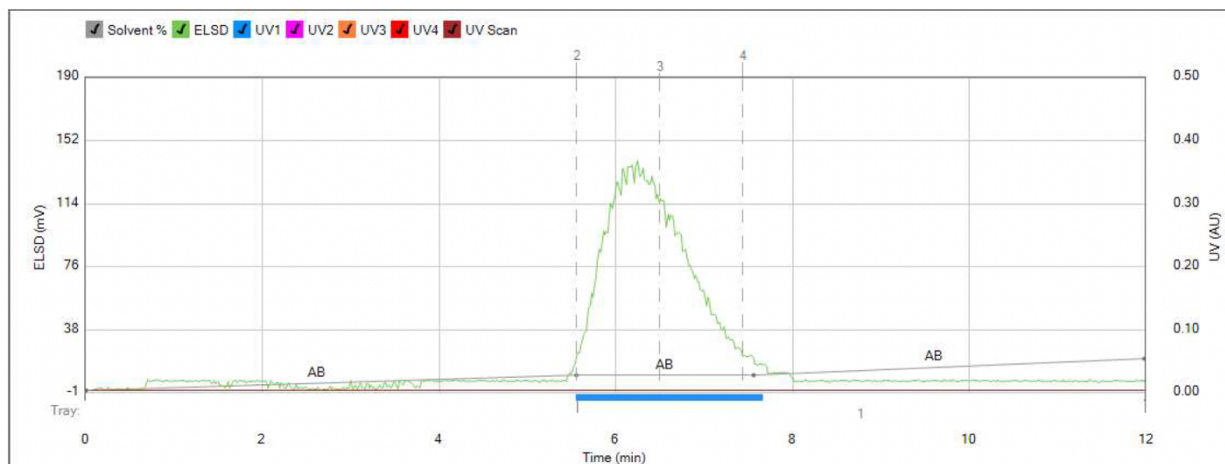
Supplementary Fig. 38 | NMR Spectrum for LC-Lipid-3.



Supplementary Fig. 39 NMR Spectrum for LC-Lipid-3 (δ 0.5-4.2 ppm region).

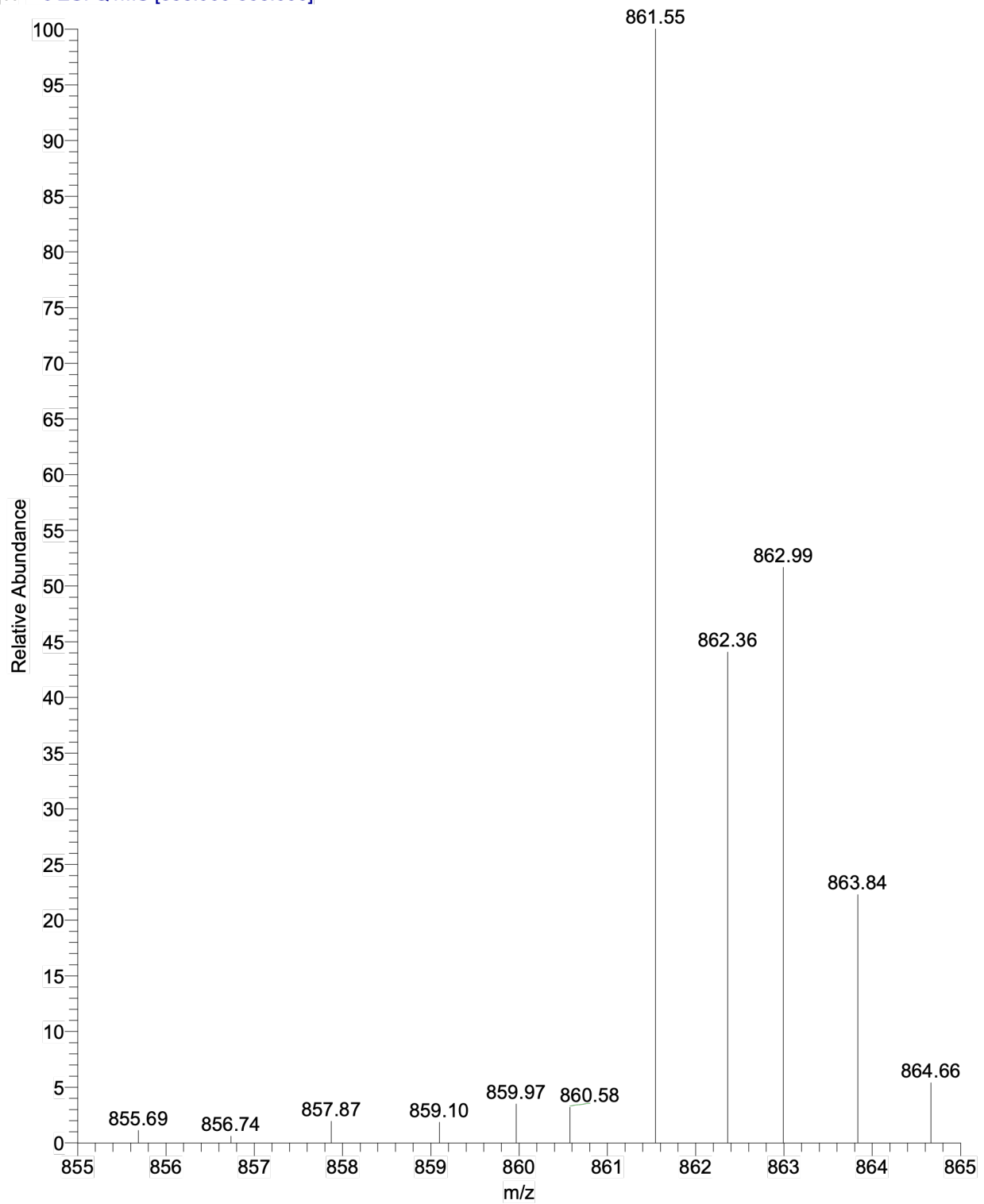


Supplementary Fig. 40 | Purification analysis of LC-lipid-3 (ELSD-HPLC).



Supplementary Fig. 41 | Purity analysis of LC-lipid-3 (ELSD-HPLC).

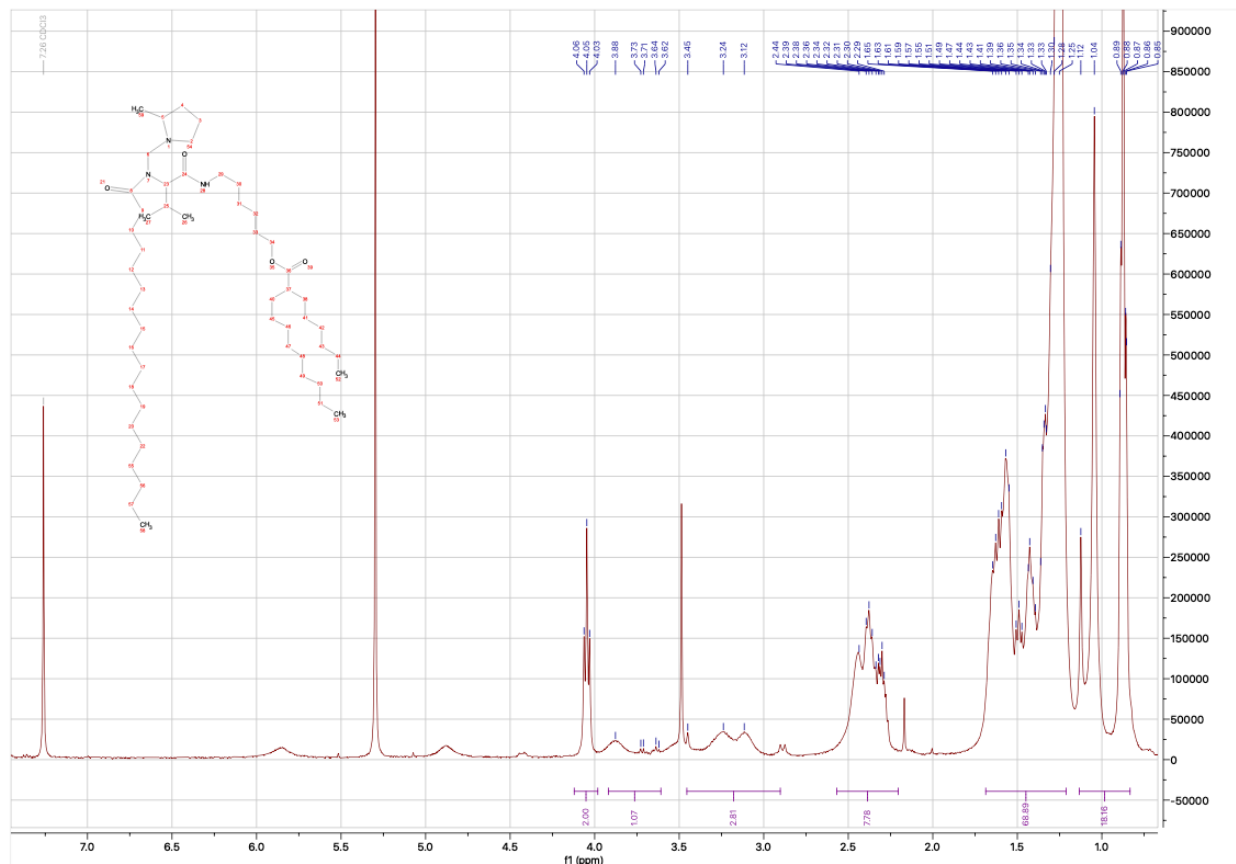
Rawdata_20260228035650 #15 RT: 0.00 AV: 1 NL: 2.40E6
T: + c ESI Q1MS [855.000-865.000]



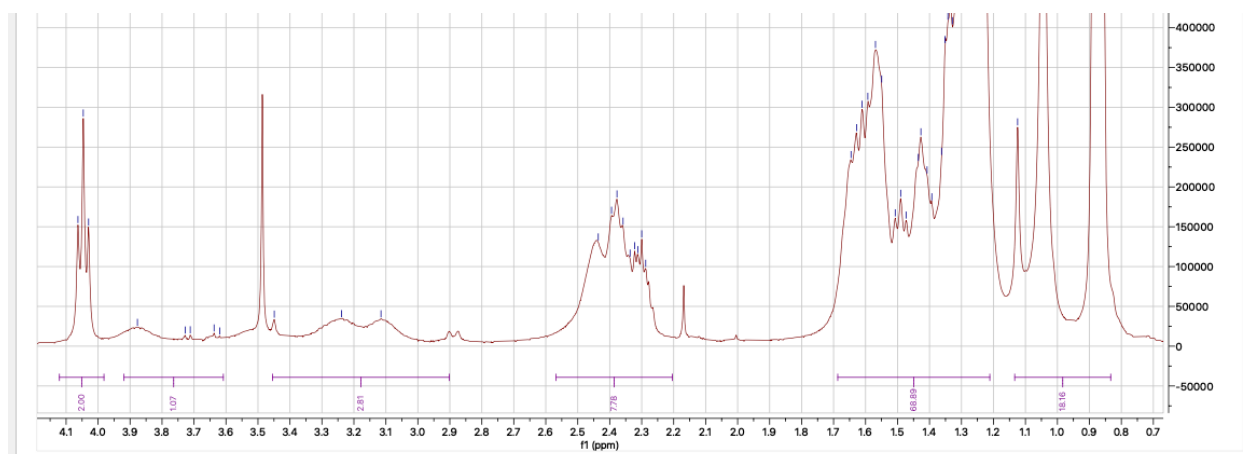
Supplementary Fig. 42 | Mass spectrometry spectrum of LC-lipid-3.

LC-Lipid-4(C₅₂H₁₀₁N₃O₄; MW: 832.40):

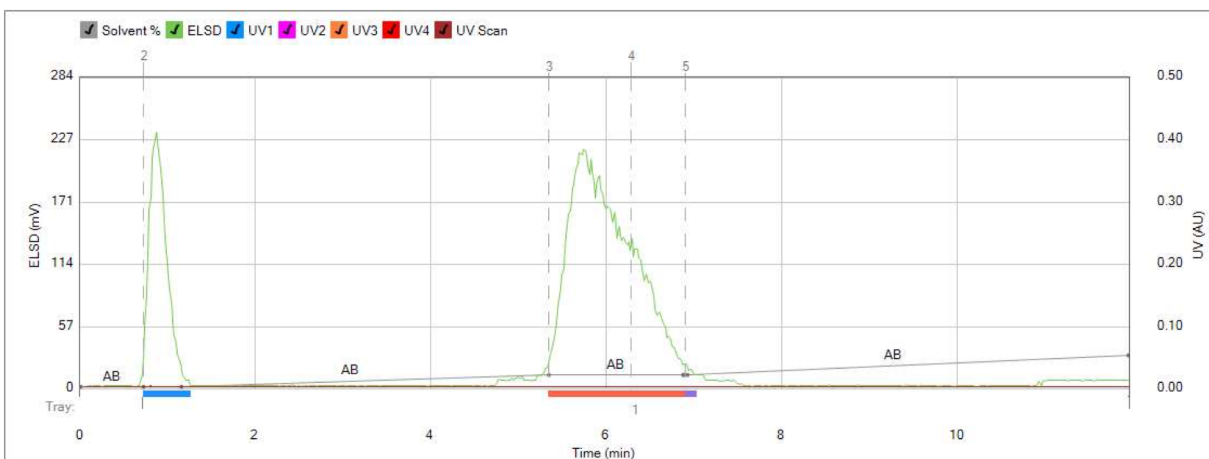
¹H NMR (400 MHz, CDCl₃) δ 4.05 (t, J = 6.6 Hz, 2H), 3.92 – 3.61 (m, 1H), 3.45 – 2.90 (m, 3H), 2.57 – 2.20 (m, 8H), 1.69 – 1.21 (m, 69H), 1.13 – 0.83 (m, 18H).



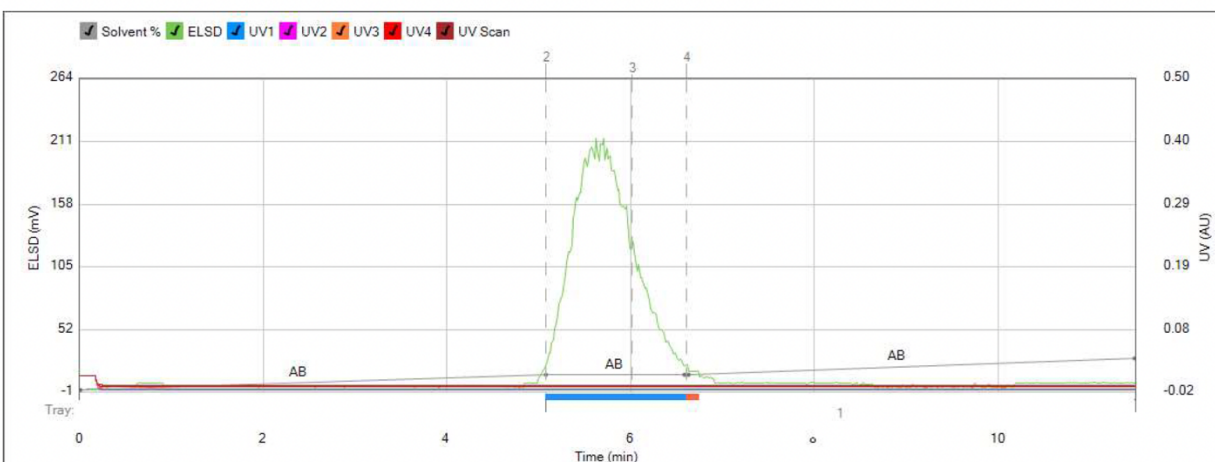
Supplementary Fig. 43 | NMR Spectrum for LC-Lipid-4.



Supplementary Fig. 44 | NMR Spectrum for LC-Lipid-4 (δ 0.5-4.2 ppm region).

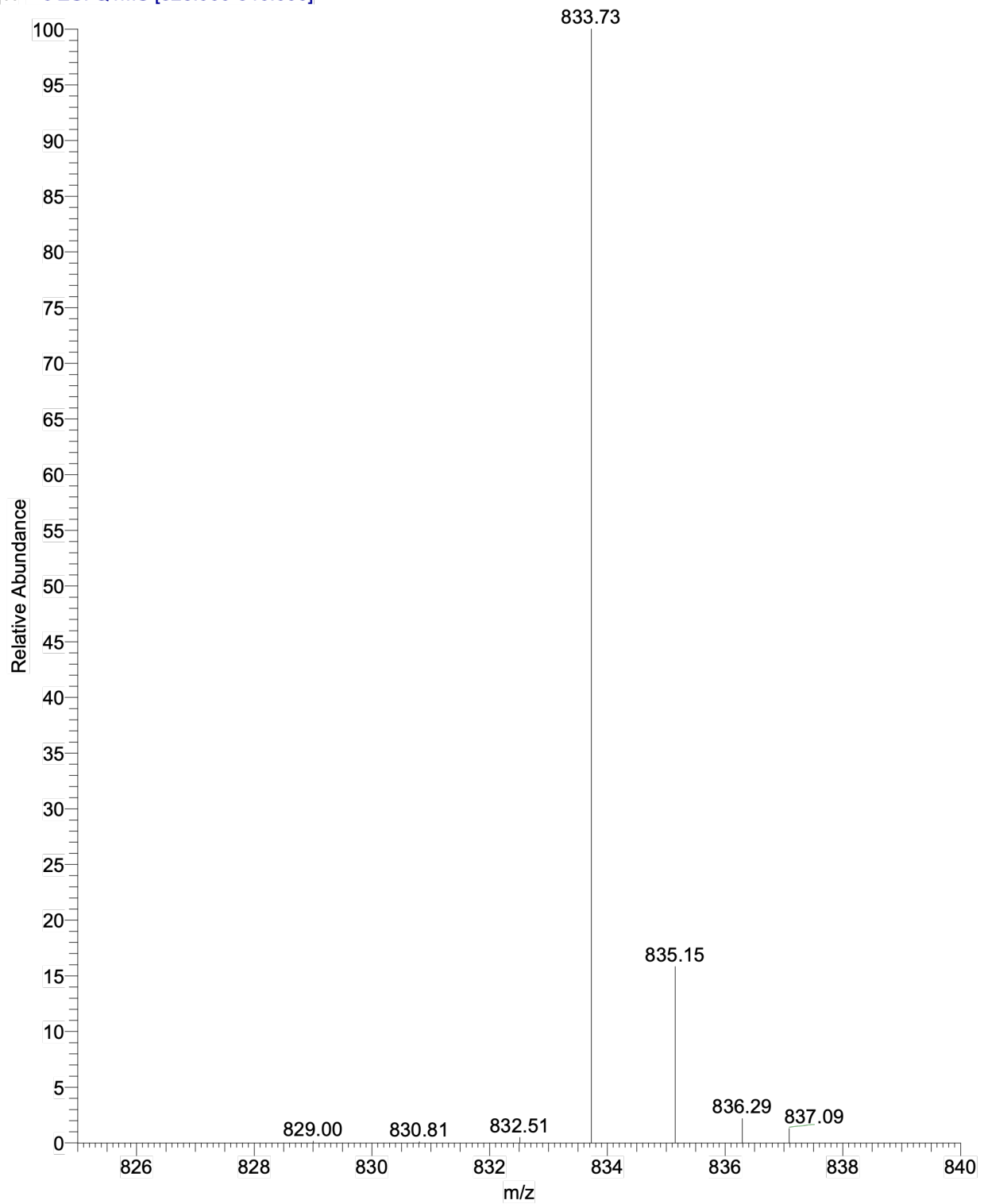


Supplementary Fig. 45 | Purification analysis of LC-lipid-4 (ELSD-HPLC).



Supplementary Fig. 46 | Purity analysis of LC-lipid-3 (ELSD-HPLC).

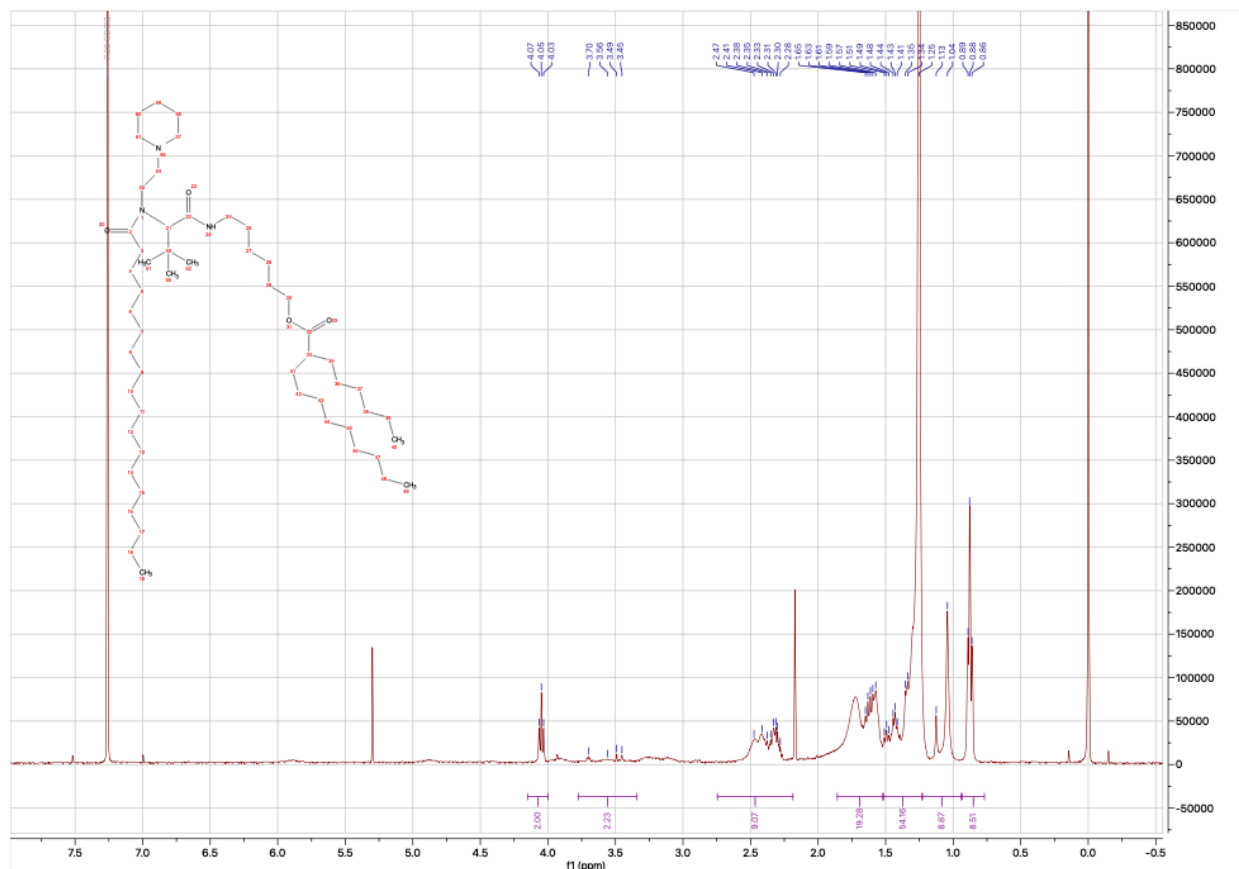
Rawdata_20260228043114 #3 RT: 0.00 AV: 1 NL: 2.57E6
T: + c ESI Q1MS [825.000-840.000]



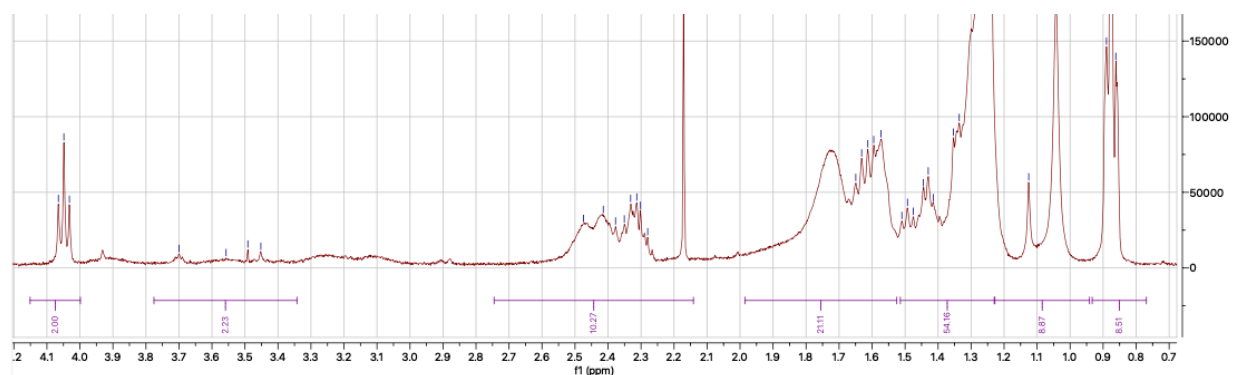
Supplementary Fig. 47 | Mass spectrometry spectrum of LC-lipid-4.

LC-Lipid-5 ($C_{54}H_{105}N_3O_4$; MW: 860.45):

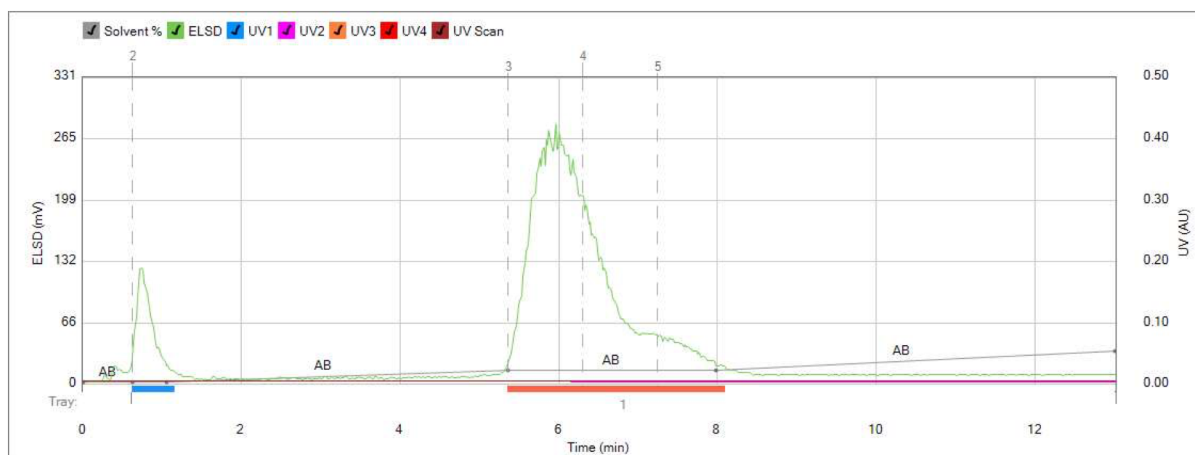
1H NMR (400 MHz, $CDCl_3$) δ 4.05 (t, $J = 6.7$ Hz, 2H), 3.78 – 3.34 (m, 2H), 2.75 – 2.14 (m, 10H), 1.99 – 1.53 (m, 21H), 1.25 (s, 54H), 1.08 (d, $J = 33.1$ Hz, 9H), 0.88 (t, $J = 5.7$ Hz, 9H).



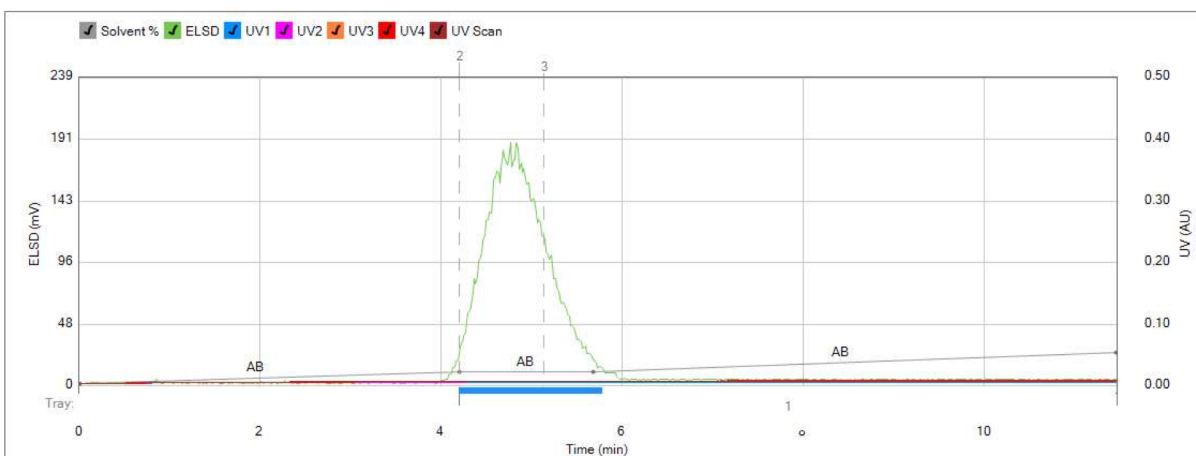
Supplementary Fig. 48 | NMR Spectrum for LC-Lipid-5.



Supplementary Fig. 49 | NMR Spectrum for LC-Lipid-5 (δ 0.5-4.2 ppm region).

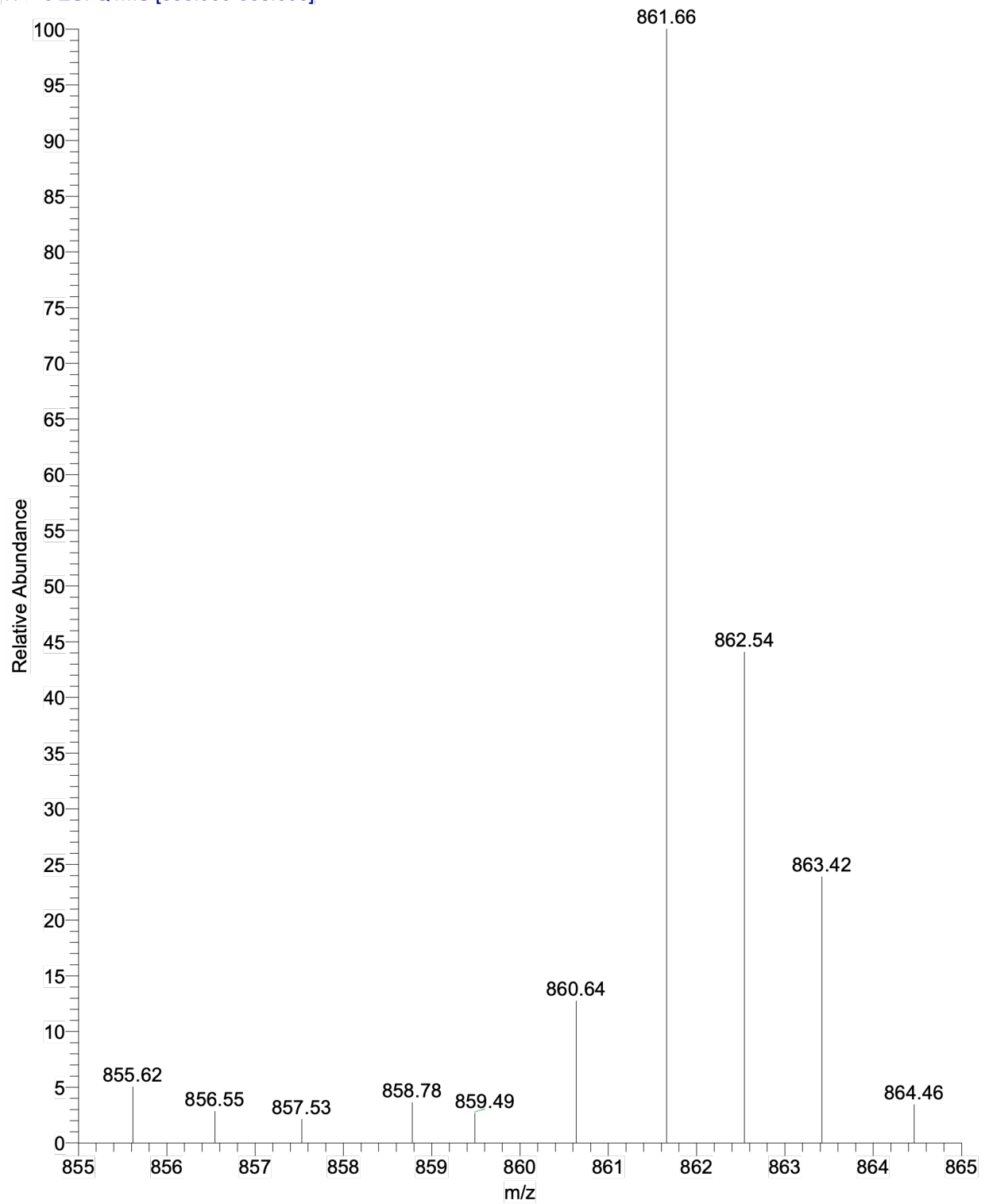


Supplementary Fig. 50 | Purification analysis of LC-lipid-5 (ELSD-HPLC).

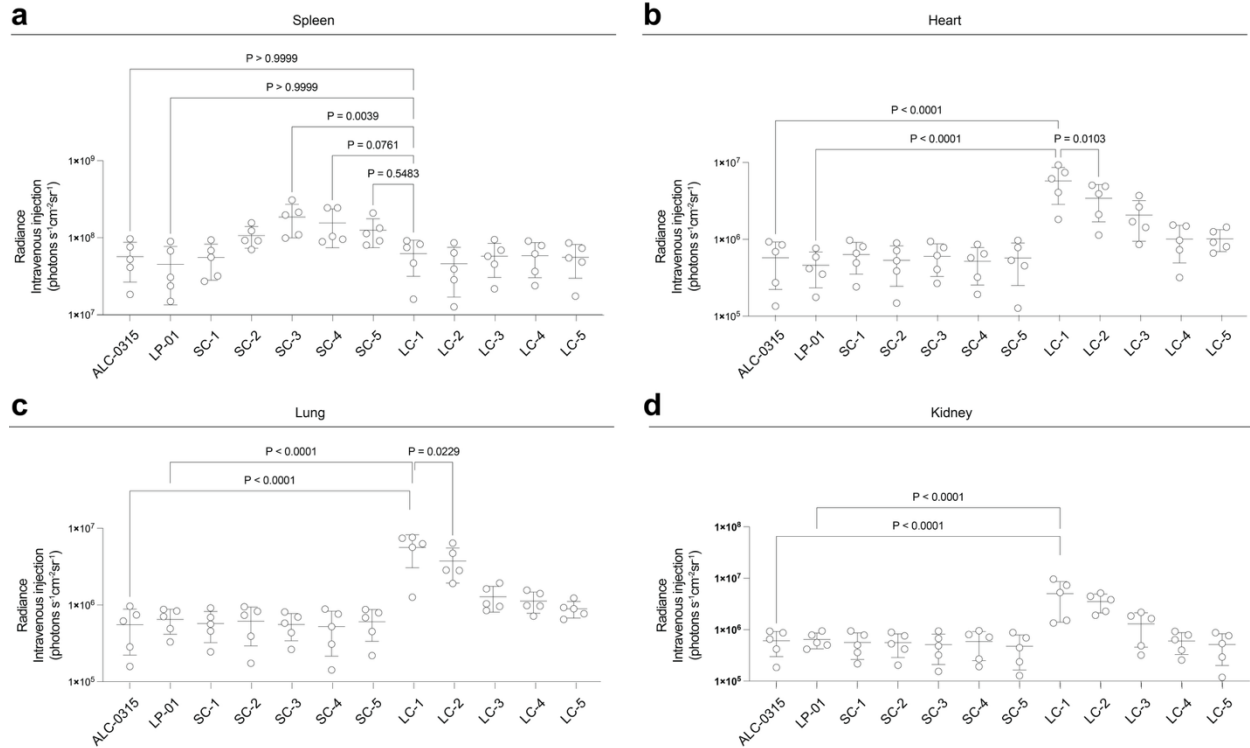


Supplementary Fig. 51 | Purity analysis of LC-lipid-5 (ELSD-HPLC).

Rawdata_20260228040027 #1685 RT: 0.34 AV: 1 NL: 1.08E6
T: + c ESI Q1MS [855.000-865.000]

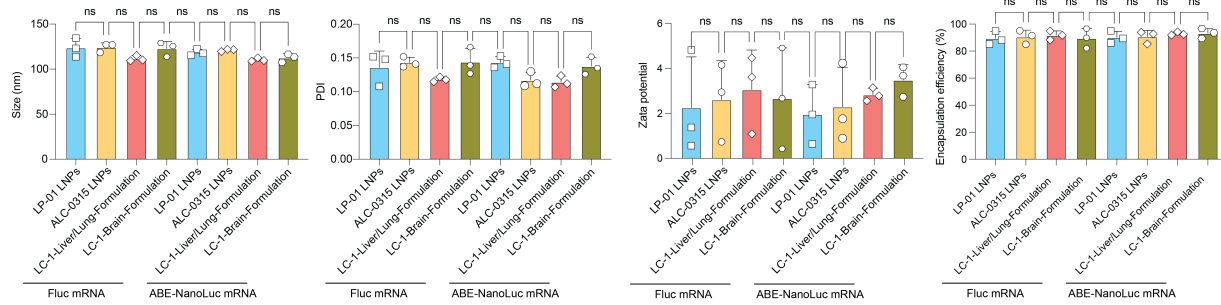


Supplementary Fig. 52 | Mass spectrometry spectrum of LC-lipid-5.

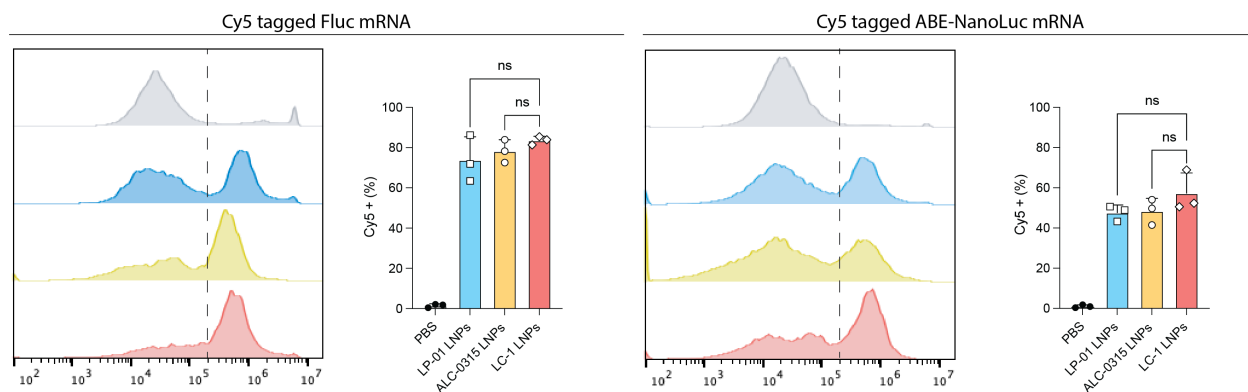


Supplementary Fig. 53 | Quantitative analysis of organ-level expression following intravenous administration of LNP formulations.

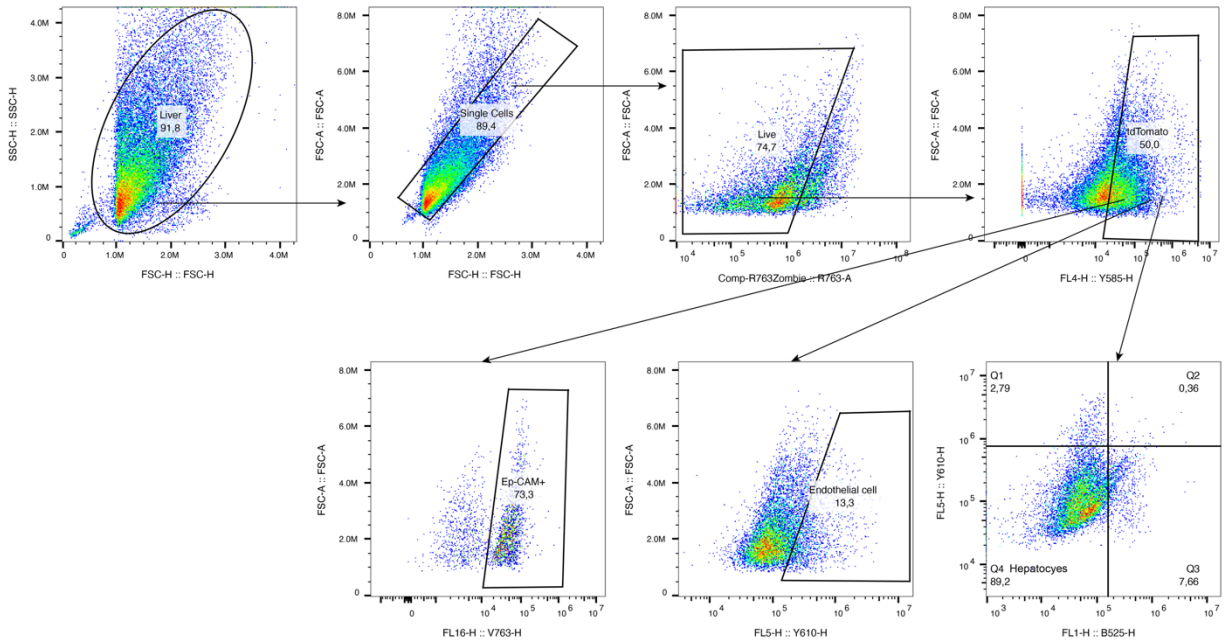
(a-d) Radiance quantification (photons s⁻¹ cm⁻² sr⁻¹) in spleen (a), heart (b), lung (c), and kidney (d) following intravenous injection of mRNA formulated in ALC-0315, LP-01, SC-series lipids (SC-1 to SC-5), and LC-series lipids (LC-1 to LC-5). (Data are presented as mean ± s.d.; n = 5 biologically independent mice per group). Statistical significance assessed by one-way ANOVA with multiple comparisons. ((b), $P_{LC-1 \text{ vs. ALC-0315}} = 7.25 \times 10^{-9}$, $P_{LC-1 \text{ vs. LP-01}} = 4.00 \times 10^{-9}$) ((c), $P_{LC-1 \text{ vs. ALC-0315}} = 4.78 \times 10^{-10}$, $P_{LC-1 \text{ vs. LP-01}} = 8.21 \times 10^{-10}$) ((d), $P_{LC-1 \text{ vs. ALC-0315}} = 3.26 \times 10^{-6}$, $P_{LC-1 \text{ vs. LP-01}} = 3.80 \times 10^{-6}$)



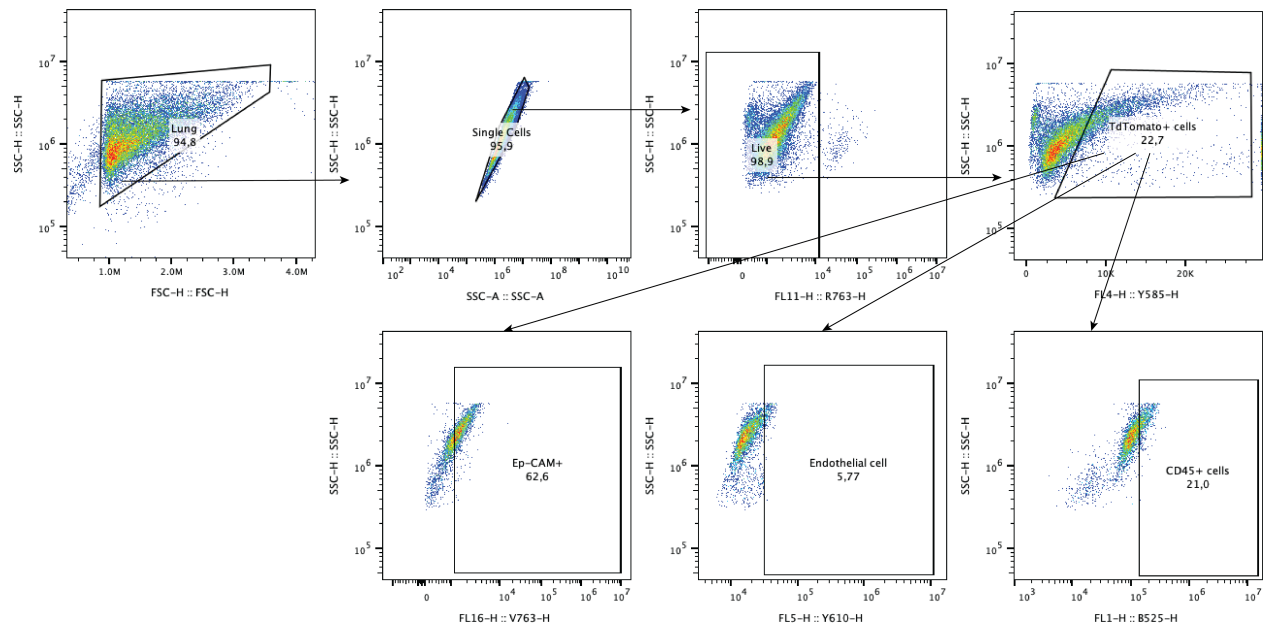
Supplementary Fig. 54 | Physicochemical characterization of LNP formulations encapsulating small- and large-cargo mRNAs. Particle size, polydispersity index (PDI), zeta potential, and encapsulation efficiency of LNPs formulated with ALC-0315, LP-01, or LC-1 ionizable lipids encapsulating small-cargo mRNA (Fluc mRNA, ~1.7 kb) or large-cargo mRNA (ABE-NanoLuc mRNA, ~5.7 kb). Data are presented as mean $\pm$ s.d. (n = 3 biological replicates). Statistical analysis was performed using one-way ANOVA; n.s., not significant.



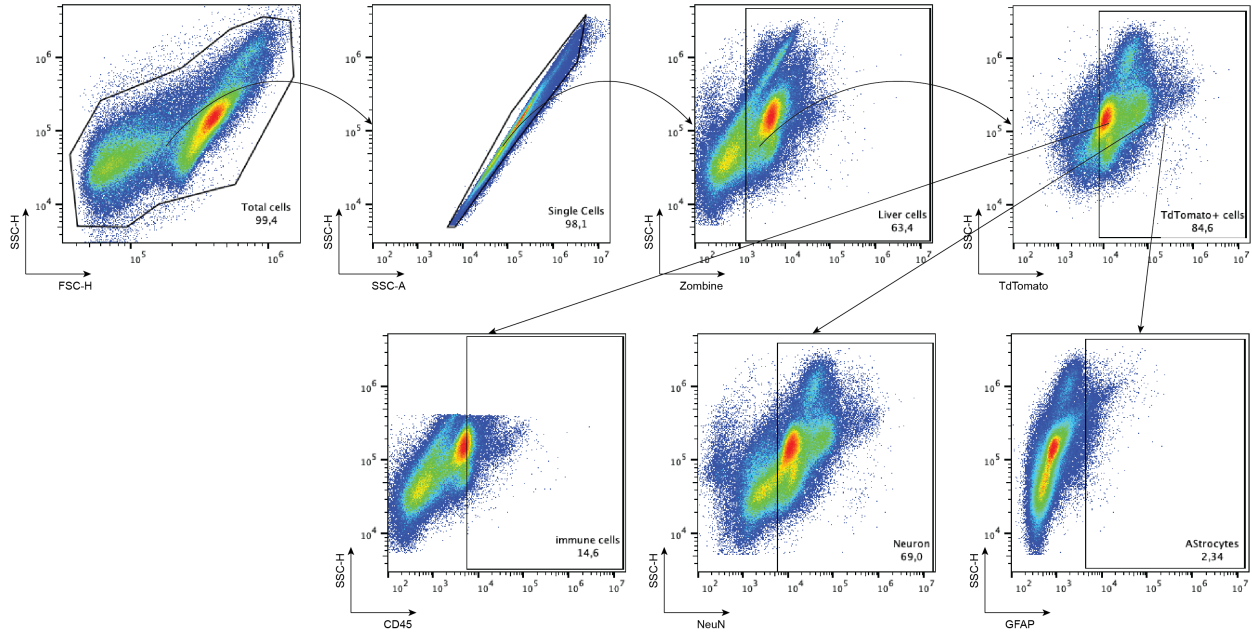
Supplementary Fig. 55 | Cellular uptake and cytosolic mRNA delivery of LNP formulations encapsulating small- and large-cargo mRNAs. Flow cytometry analysis of Cy5-labeled mRNA delivery by LNPs formulated with ALC-0315, LP-01, or LC-1 lipids encapsulating small-cargo mRNA (Fluc mRNA, ~1.7 kb) or large-cargo mRNA (ABE-NanoLuc mRNA, ~5.7 kb). Data are presented as mean $\pm$ s.d. ($n = 3$ biological replicates). Statistical significance was assessed by one-way ANOVA (ns, not significant).



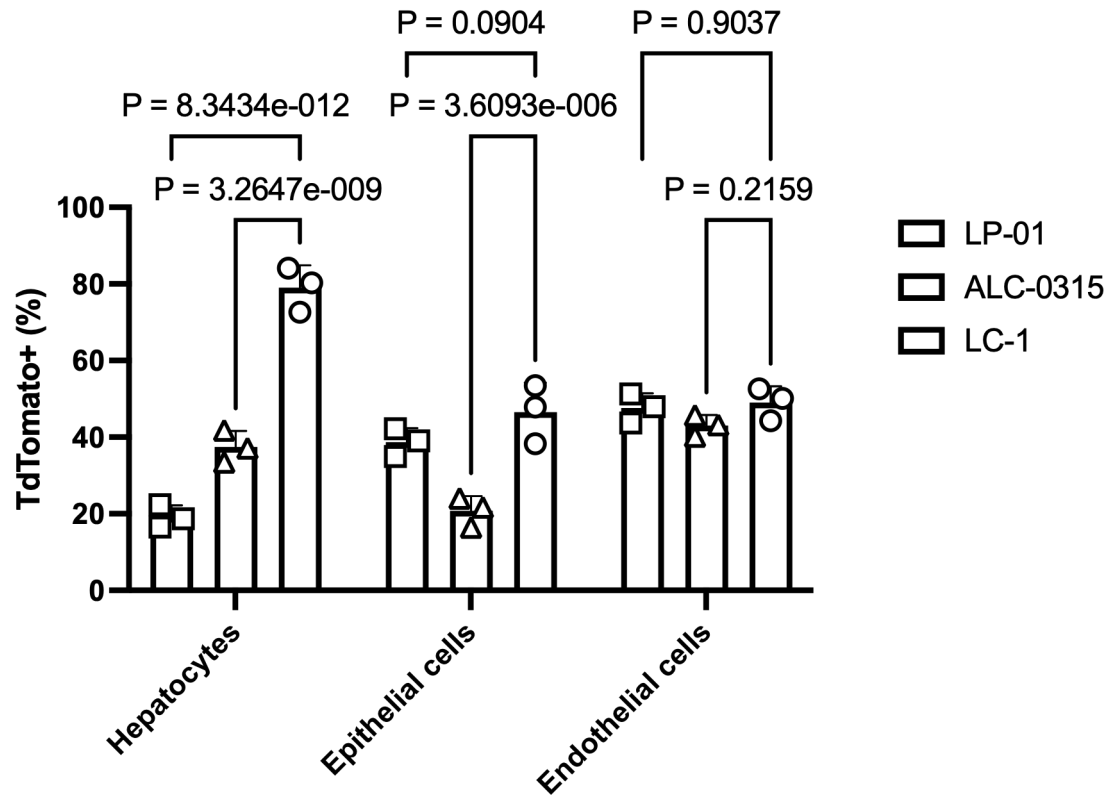
Supplementary Fig. 56 | Representative flow cytometry gating strategy for identifying epithelial cells, endothelial cells, hepatocytes, and TdTomato⁺ cells in liver tissues of Ai9 mice after intravenous administration of LNPs loading with Cas9/sgTOM mRNA (1 mg/kg).



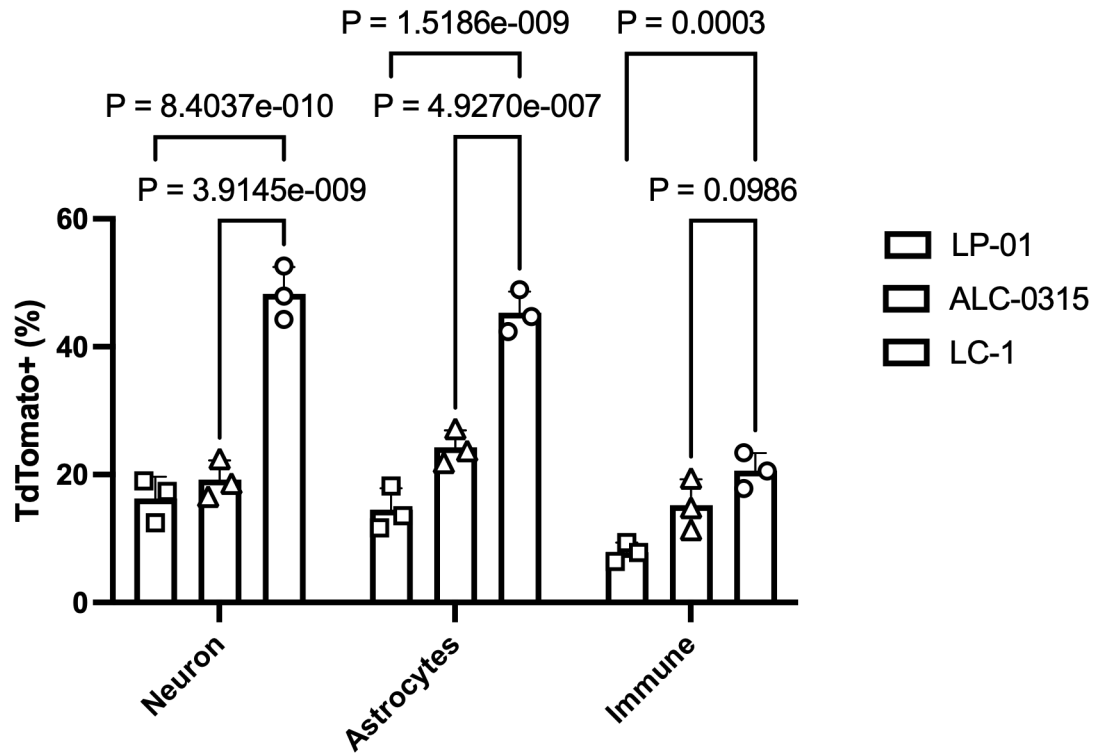
Supplementary Fig. 57 | Representative flow cytometry gating strategy for identifying epithelial cells, endothelial cells, immune cells, and TdTomato⁺ cells in lung tissues of Ai9 mice after intratracheal administration of LNPs loading with Cas9/sgTOM mRNA (0.5 mg/kg).



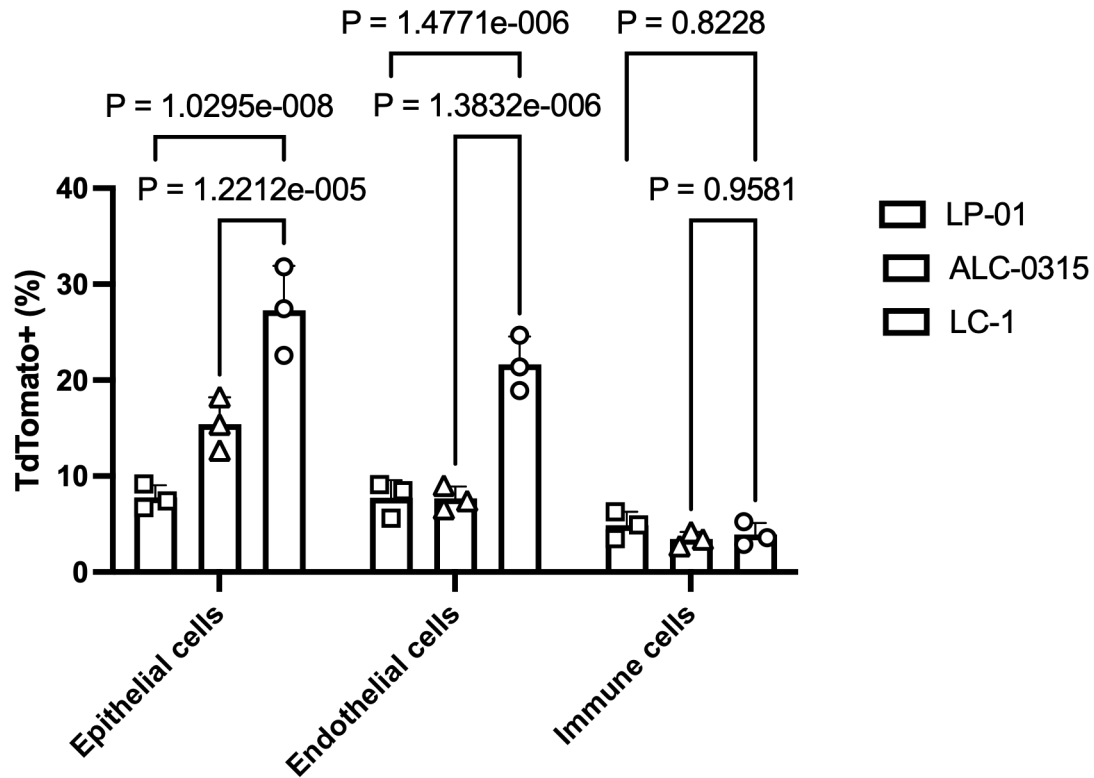
Supplementary Fig. 58 | Representative flow cytometry gating strategy for identifying neuron cells, astrocytes cells, immune cells, and TdTomato⁺ cells in brain tissues of Ai9 mice after intrathecal administration of LNPs loading with Cas9/sgTOM mRNA (0.1 mg/kg).



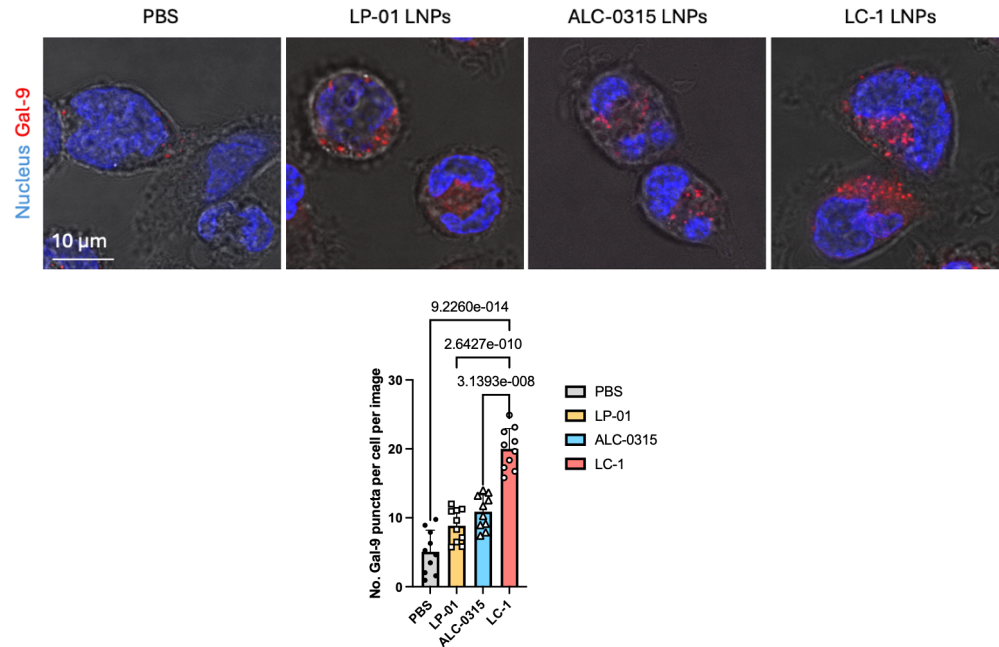
Supplementary Fig. 59 | Quantification of TdTomato⁺ cells percentages in hepatocytes, epithelial cells, and endothelial cells from Cas9 edited liver tissues from Ai9 mice after intravenous administration of Cas9/sgTOM LNPs (1 mg/kg). Each dot represents one biological replicate (n=3 biological replicates). Data are shown as mean $\pm$ s.d. Statistical significance was determined by one-way ANOVA with multiple comparisons.



Supplementary Fig. 60 | Quantification of TdTomato⁺ cells percentages in neuron cells, astrocytes cells, and immune cells from Cas9 edited brain tissues from Ai9 mice after intrathecal administration of Cas9/sgTOM LNPs (0.1 mg/kg). Each dot represents one biological replicate (n=3 biological replicates). Data are shown as mean $\pm$ s.d. Statistical significance was determined by one-way ANOVA with multiple comparisons.

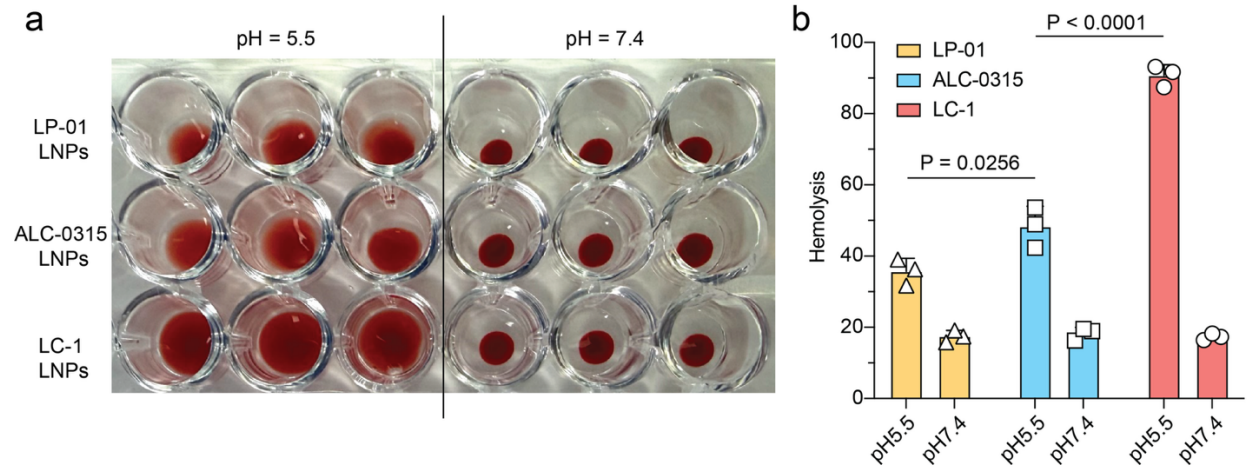


Supplementary Fig. 61 | Quantification of TdTomato⁺ cells percentages in epithelial cells, endothelial cells and immune cells from Cas9 edited lung tissues from Ai9 mice after intratracheal administration of Cas9/sgTOM LNPs (0.5 mg/kg). Each dot represents one biological replicate (n=3 biological replicates). Data are shown as mean $\pm$ s.d. Statistical significance was determined by one-way ANOVA with multiple comparisons.



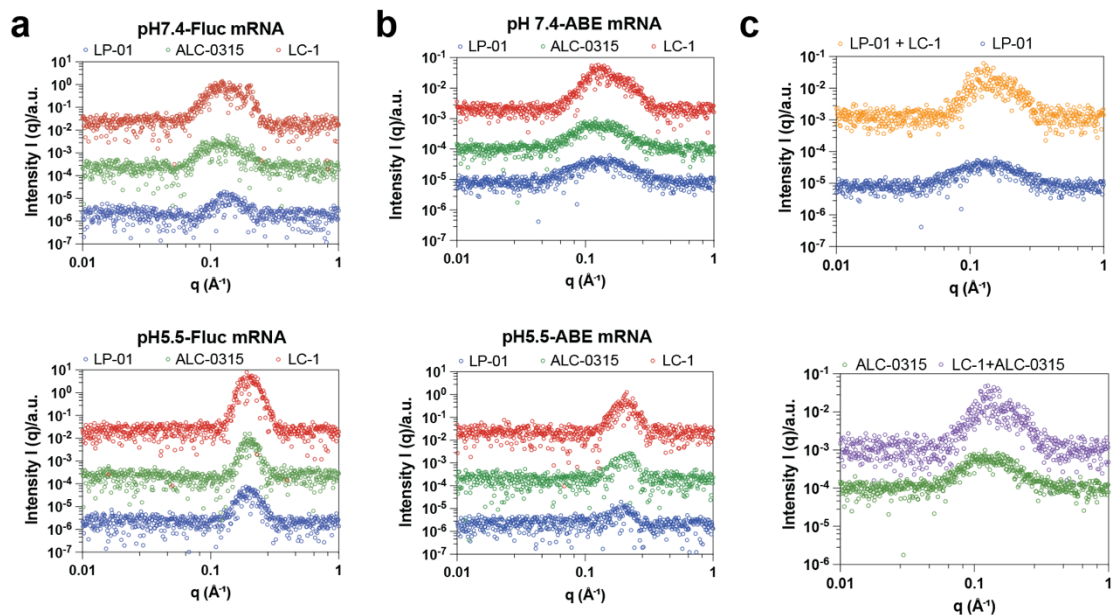
Supplementary Fig. 62 | LC-1 LNPs induce enhanced endosomal disruption as measured by Gal-9 recruitment.

Representative confocal images of Galectin-9 (Gal-9) puncta formation in cells treated with PBS, LP-01 LNPs, ALC-0315 LNPs, or LC-1 LNPs. Nuclei are stained in blue and Gal-9 puncta are shown in red. Quantification of Gal-9 puncta per cell is shown on the right. Data are presented as mean $\pm$ s.d. (n = 10 biological replicates). Statistical significance was determined by one-way ANOVA.

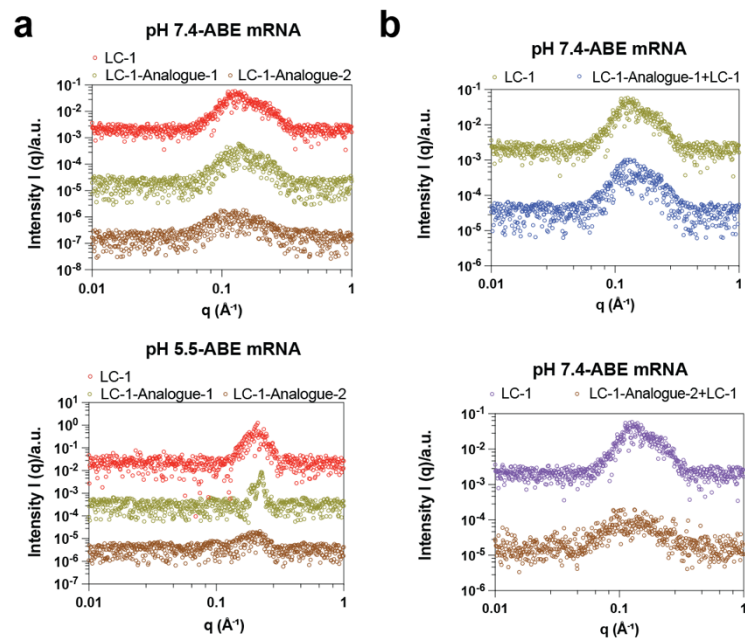


Supplementary Fig. 63 | pH-dependent membrane-disruptive activity of LC-1 LNPs assessed by hemolysis assay.

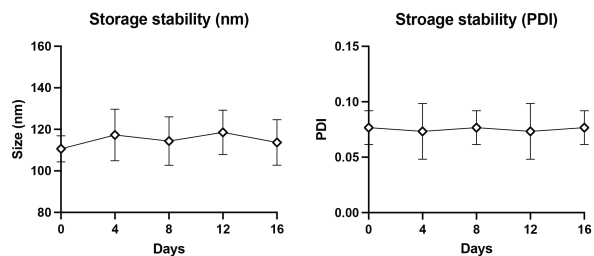
(a) Representative images of red blood cell suspensions following incubation with LP-01, ALC-0315, or LC-1 LNPs at pH 5.5 or pH 7.4. Increased hemoglobin release at acidic pH indicates membrane disruption. (b) Quantification of percent hemolysis under each condition. Data are presented as mean $\pm$ s.d. ($n = 3$ biological replicates). Statistical significance was determined by one-way ANOVA. ($P_{\text{LC-1 vs. ALC-0315}} = 4.54 \times 10^{-5}$)



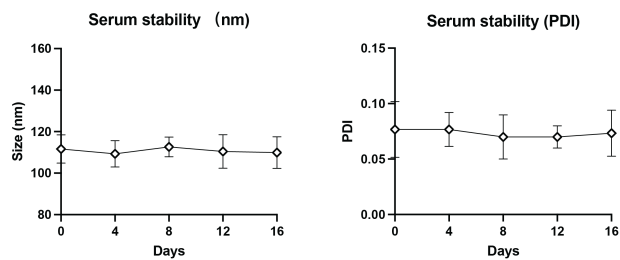
Supplementary Fig. 64 | Small-angle X-ray scattering (SAXS) profiles of LP-01, ALC-0315, and LC-1 LNPs encapsulating Fluc mRNA (**a**) or ABE mRNA (**b**) at pH 7.4 and pH 5.5. (**c**) SAXS profiles show that incorporation of LC-1 into LP-01 or ALC-0315 formulations alters internal lipid organization, suggesting a cooperative effect on LNP structural packing.



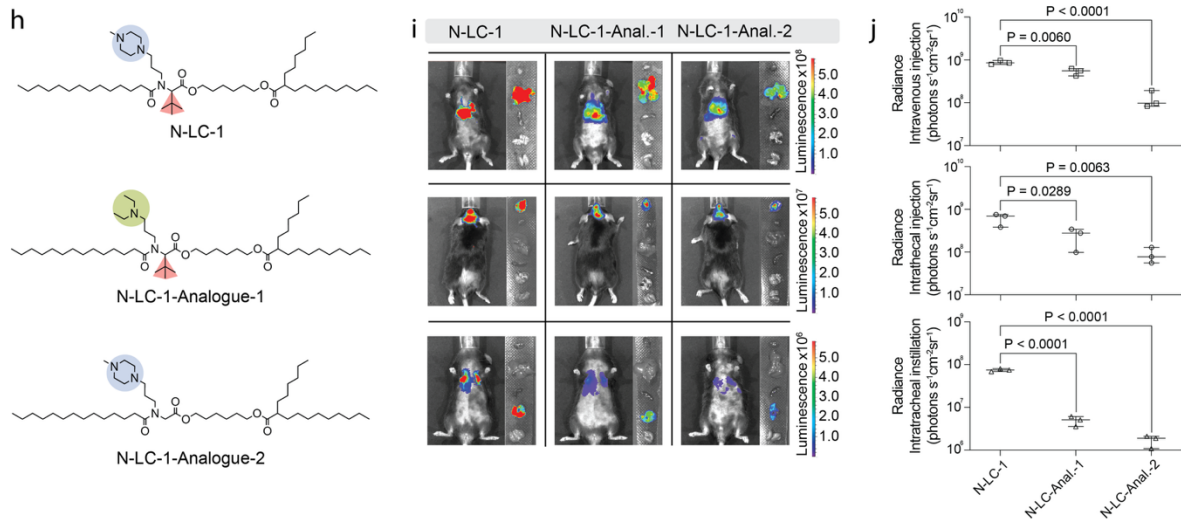
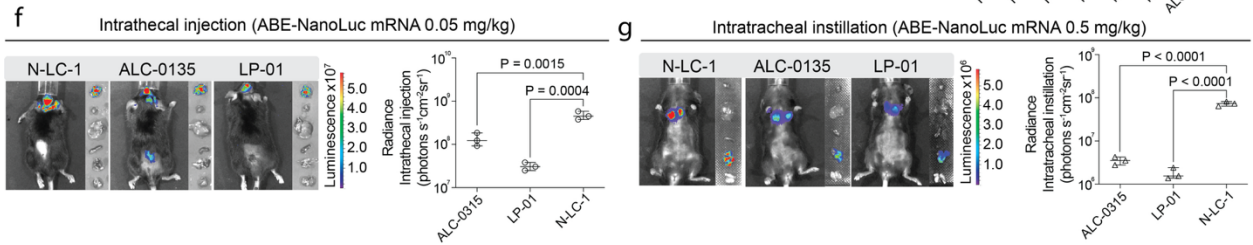
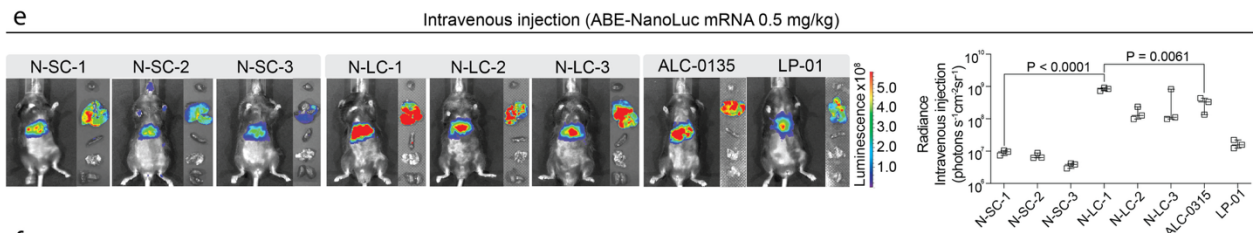
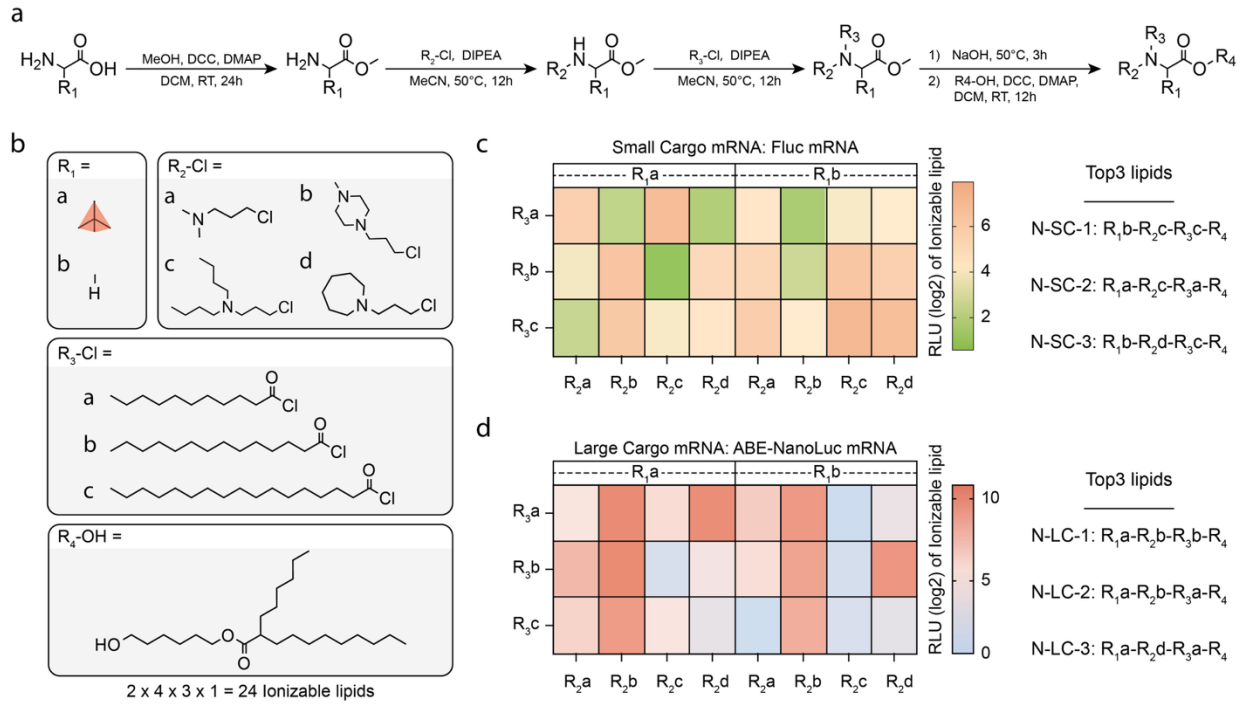
Supplementary Fig. 65 | (a) SAXS profiles and representative cryo-TEM images with Fast Fourier Transform (FFT) patterns of LC-1, LC-1-Analogue-1, or LC-1-Analogue-2 at pH 7.4 and pH 5.5. (b) Corresponding SAXS characterization of LC-Lipid analogues and their combinations with LC-1.



Supplementary Fig. 66 | Storage stability of LC-1 LNP formulations. Hydrodynamic diameter (left) and polydispersity index (PDI, right) of LC-1 LNPs monitored over 14 days under storage conditions (4°C). Data are presented as mean $\pm$ s.d. (n = 3 biological replicates).



Supplementary Fig. 67 | Serum stability of LC-1 LNP formulations. Hydrodynamic diameter (left) and polydispersity index (PDI, right) of LC-1 LNPs following incubation in serum over 14 days. Data are presented as mean $\pm$ s.d. (n = 3 biological replicates).

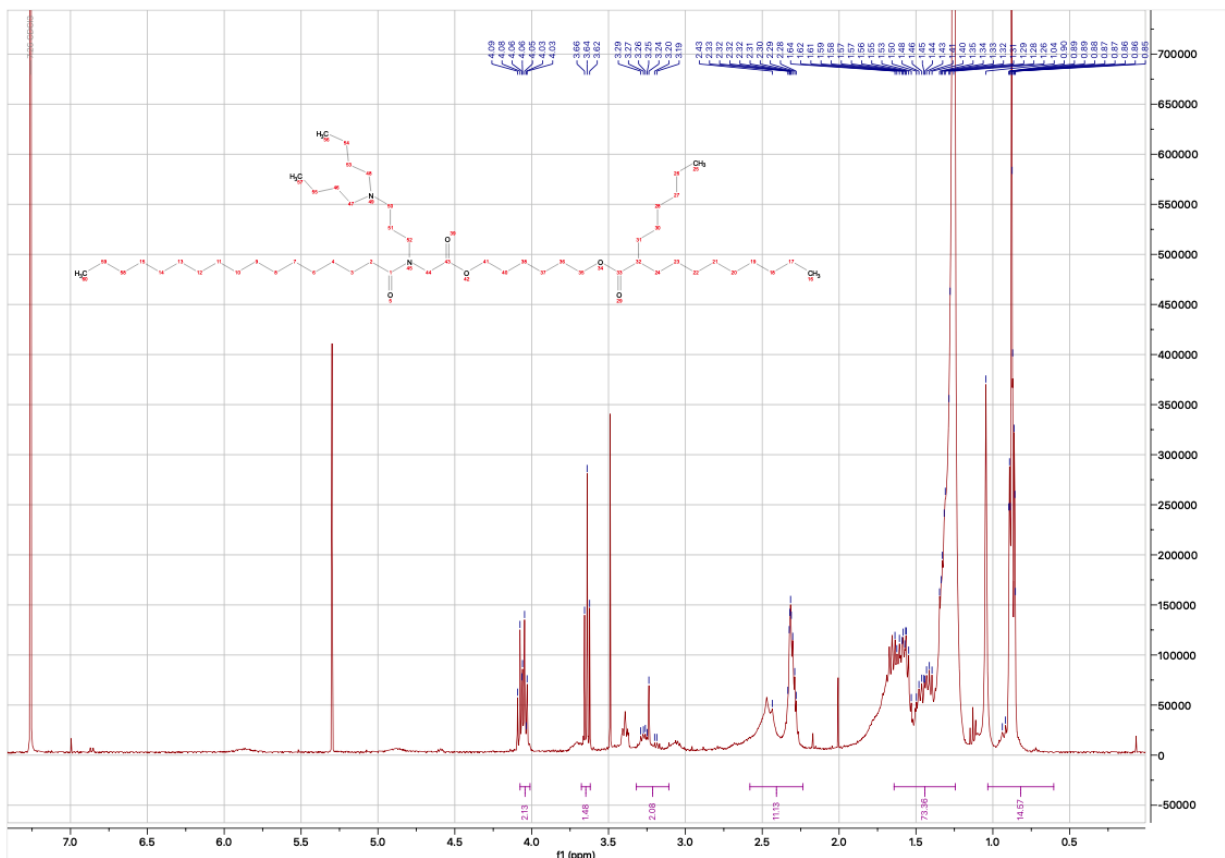


Supplementary Fig. 68 | Large-cargo-informed screening using a conventionally synthesized ionizable lipid library.

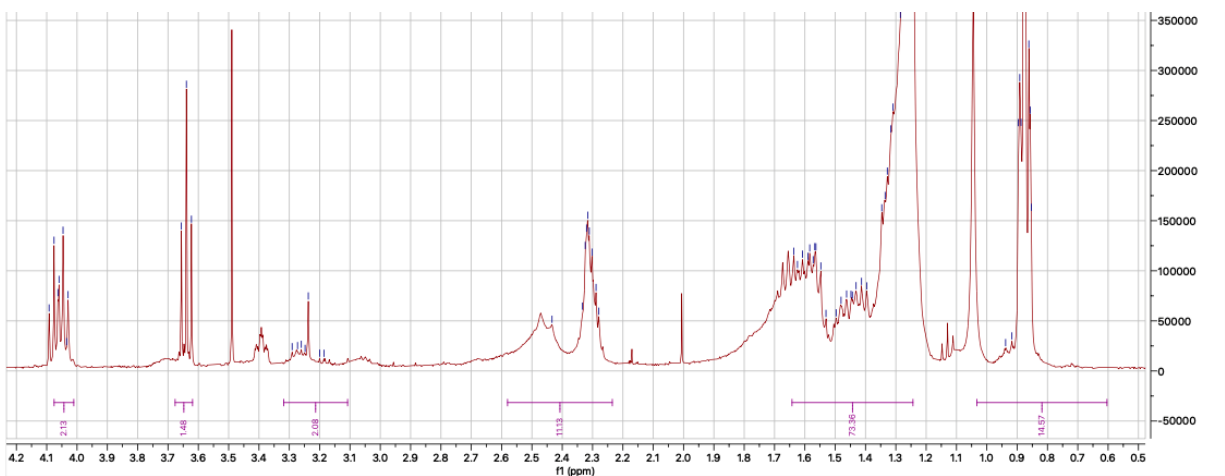
(a) Schematic illustration of the stepwise synthetic route used to generate a structurally diverse ionizable lipid library via conventional multi-step chemistry. (b) Representative structural modules incorporated into the library. (c) Heatmap summarizing transfection performance of the synthesized lipids formulated with small-cargo Fluc mRNA. (d) Corresponding performance with large-cargo ABE-NanoLuc mRNA, revealing distinct structure–activity relationships between small- and large-cargo screening. Top-performing lipids identified from each screening strategy are indicated. (e–g) Representative in vivo bioluminescence imaging and quantification following intravenous, intrathecal, and intratracheal administration of ABE-NanoLuc mRNA formulated in selected lipids and benchmark controls (ALC-0315 and LP-01). (h) Chemical structures of representative lead lipids and structurally related analogues generated from the conventional synthetic route. (i, j) In vivo delivery performance and quantification of selected lipids and analogues, demonstrating that large-cargo screening consistently identifies superior candidates across administration routes. Data are presented as mean $\pm$ s.d. (n = 3 biological replicates). statistical significance was determined by one-way ANOVA with multiple comparisons. ((e), $P_{\text{N-LC-1 vs. N-SC-1}} = 8.10 \times 10^{-5}$) ((g), $P_{\text{N-LC-1 vs. ALC-0315}} = 5.54 \times 10^{-6}$, $P_{\text{LC-1 vs. LP-01}} = 4.79 \times 10^{-6}$) ((j), Intravenous injection, $P_{\text{N-LC-1 vs. N-LC-Anal-2}} = 8.36 \times 10^{-5}$, Intratracheal injection, $P_{\text{N-LC-1 vs. N-LC-Anal-2}} = 4.76 \times 10^{-7}$, $P_{\text{LC-1 vs. N-LC-Anal-1}} = 3.63 \times 10^{-7}$)

N-SC-Lipid-1 (C₅₃H₁₀₄N₂O₅; MW: 849.42):

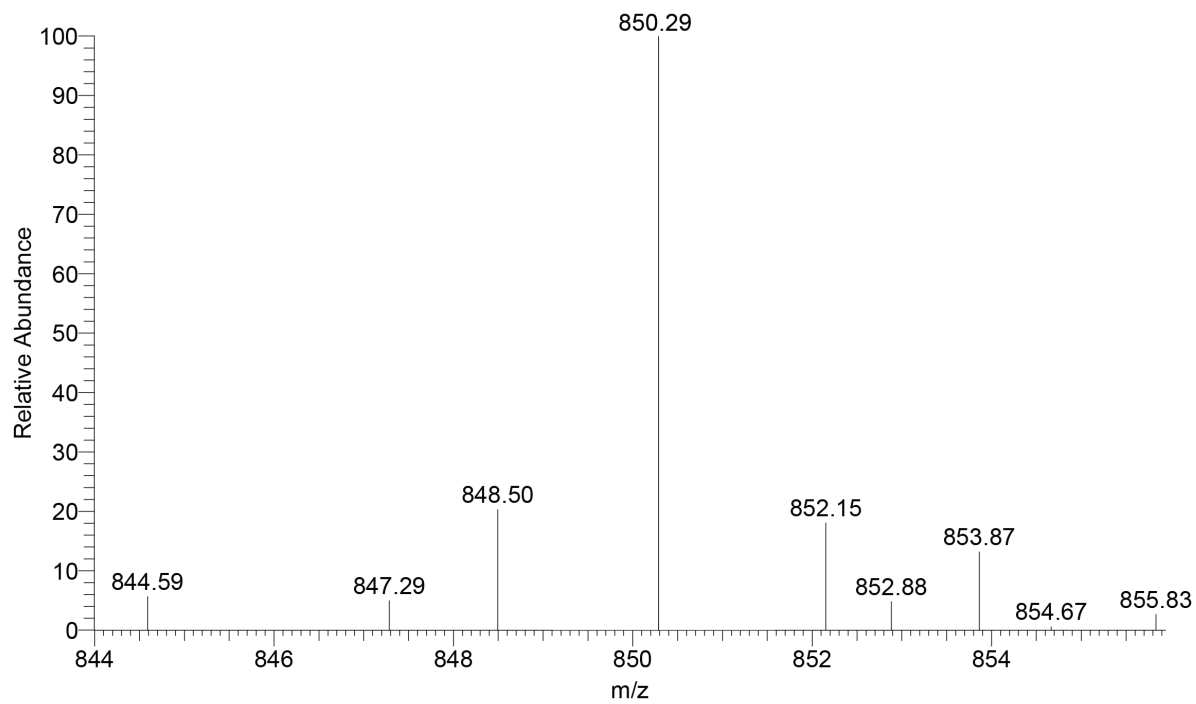
¹H NMR (400 MHz, CDCl₃) δ 4.08 – 4.01 (m, 2H), 3.64 (t, J = 6.6 Hz, 1H), 3.32 – 3.11 (m, 2H), 2.58 – 2.23 (m, 11H), 1.64 – 1.24 (m, 73H), 1.03 – 0.60 (m, 15H)



Supplementary Fig. 69 | NMR Spectrum for N-SC-Lipid-1.



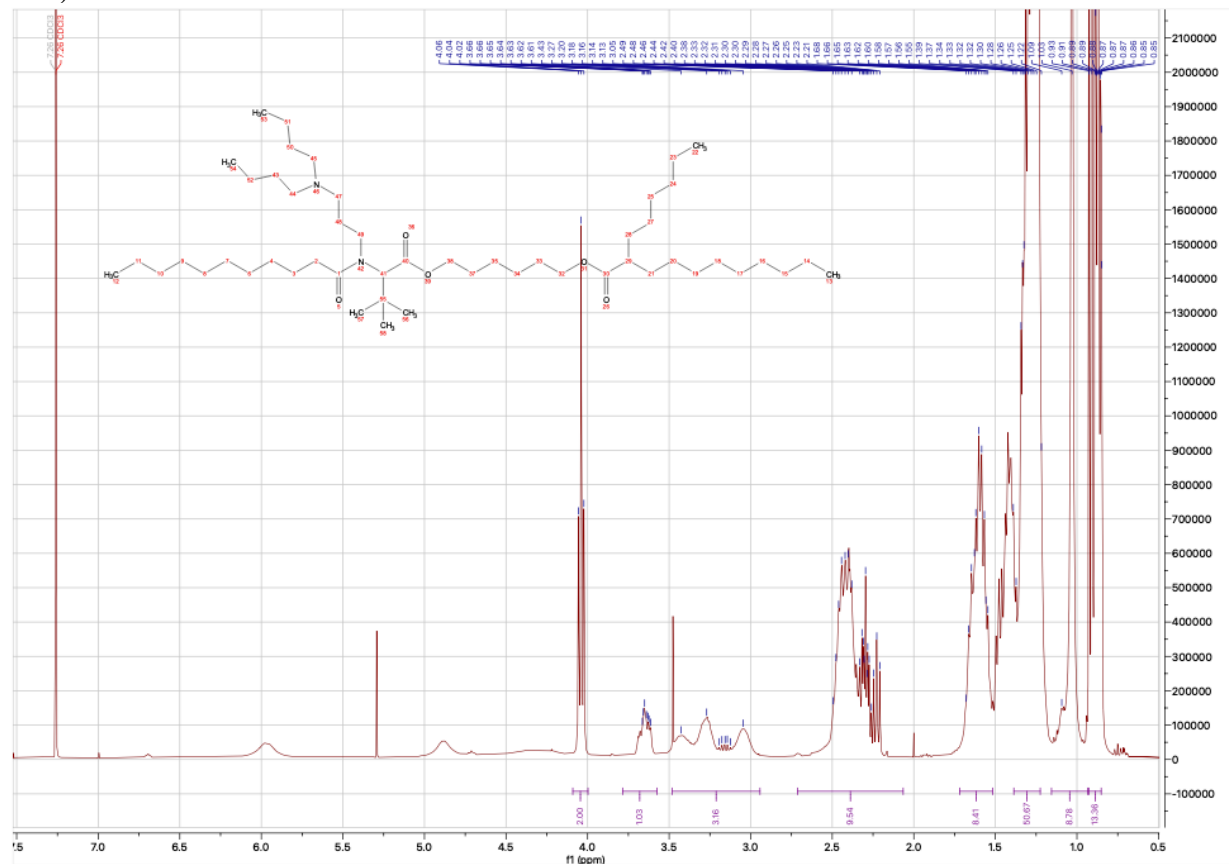
Supplementary Fig. 70 | NMR Spectrum for N-SC-Lipid-1 (δ 0-4.2 ppm region).



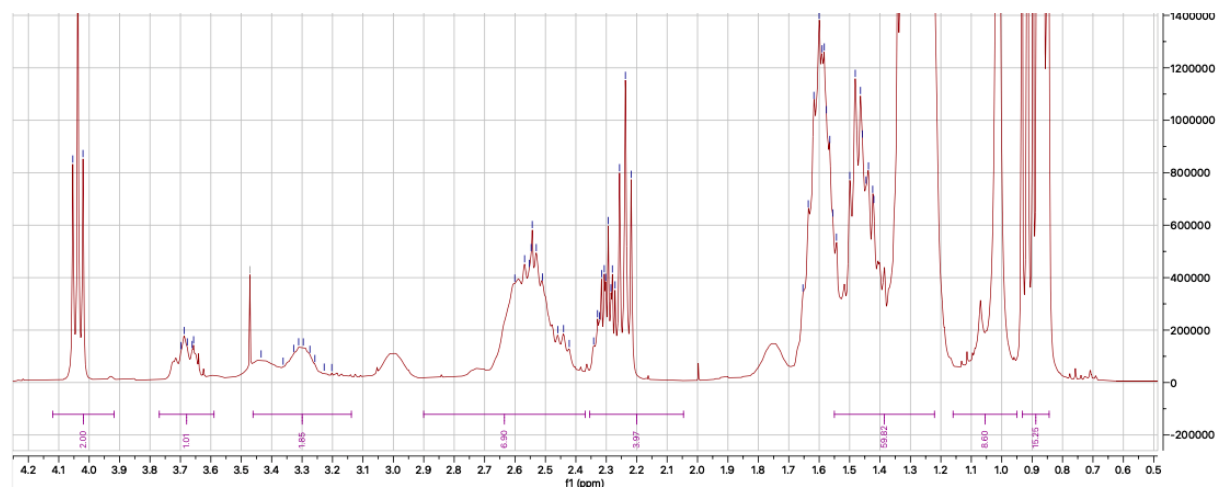
Supplementary Fig. 71 | Mass spectrometry spectrum of N-SC-Lipid-1.

N-SC-Lipid-2 ($C_{51}H_{100}N_2O_5$; MW: 821.37):

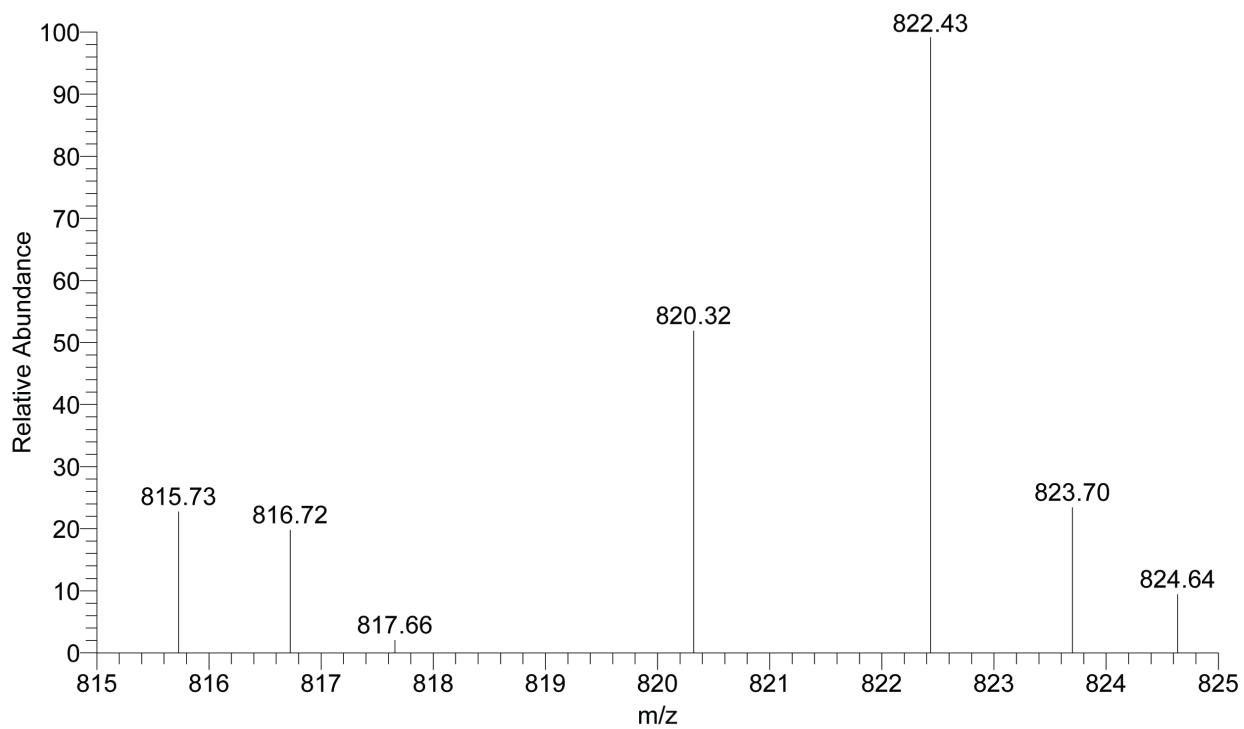
1H NMR (400 MHz, $CDCl_3$) δ 4.04 (t, $J = 6.7$ Hz, 2H), 3.77 – 3.59 (m, 1H), 3.46 – 3.14 (m, 2H), 2.90 – 2.37 (m, 7H), 2.35 – 2.05 (m, 4H), 1.55 – 1.22 (m, 60H), 1.01 (s, 9H), 0.93 – 0.84 (m, 15H).



Supplementary Fig. 72 | NMR Spectrum for N-SC-Lipid-2.



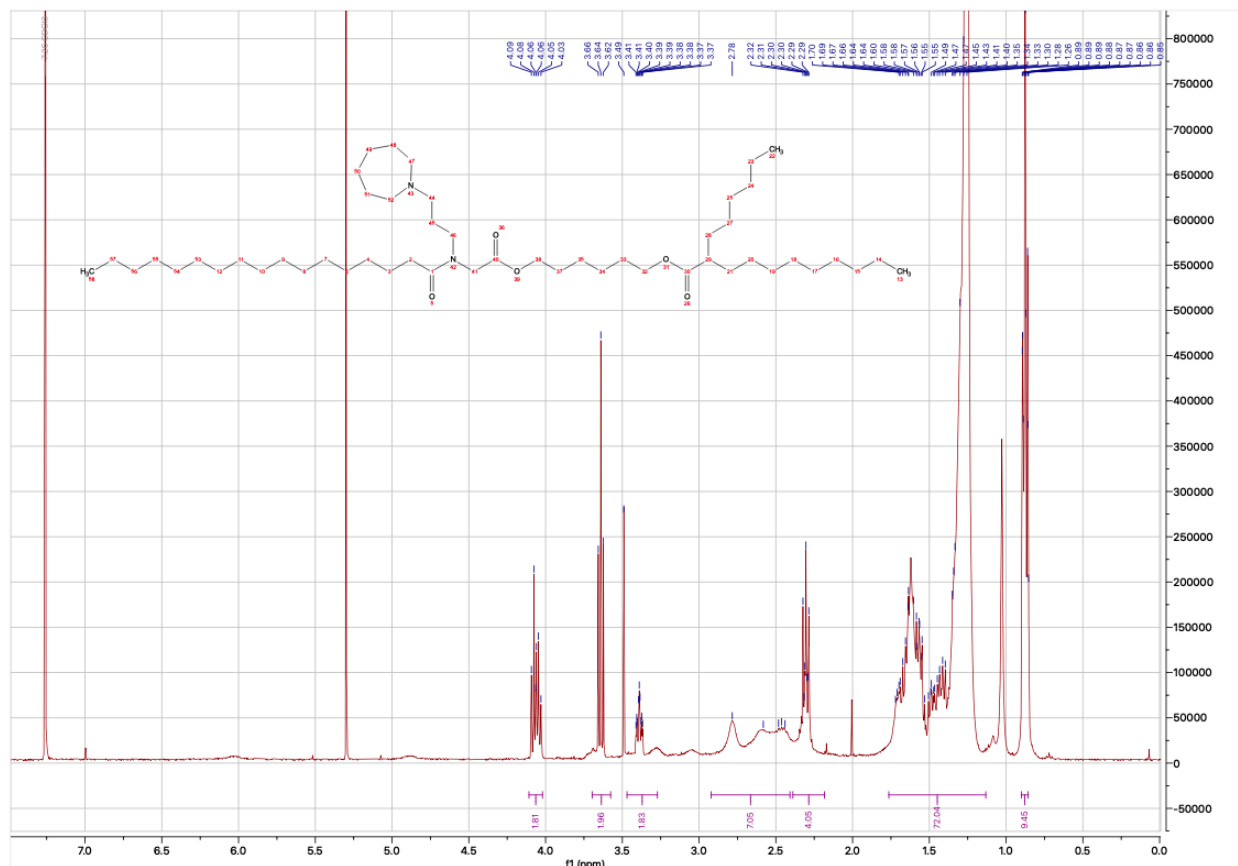
Supplementary Fig. 73 | NMR Spectrum for N-SC-Lipid-2 (δ 0.5-4.2 ppm region).



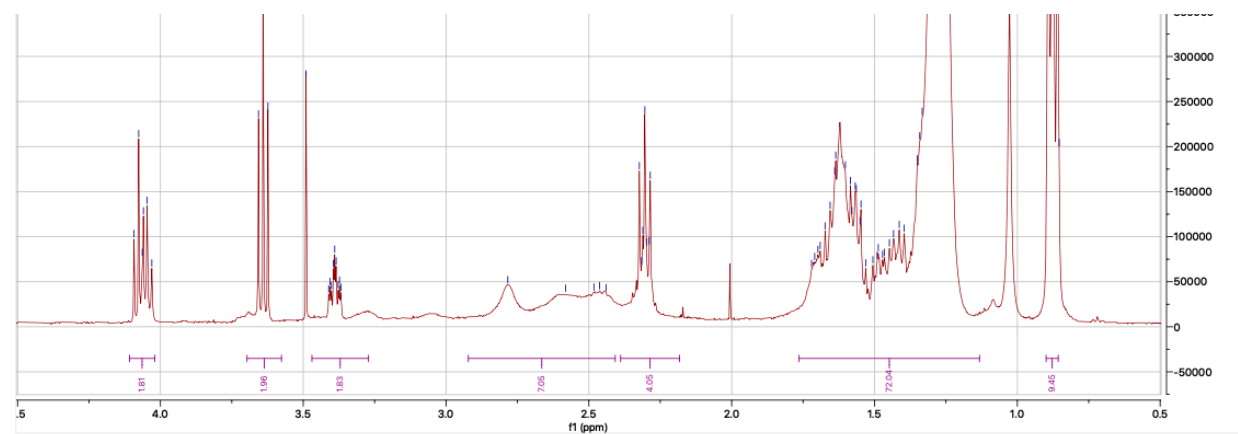
Supplementary Fig. 74 | Mass spectrometry spectrum of N-SC-Lipid-2.

N-SC-Lipid-3 ($C_{51}H_{98}N_2O_5$; MW: 819.35):

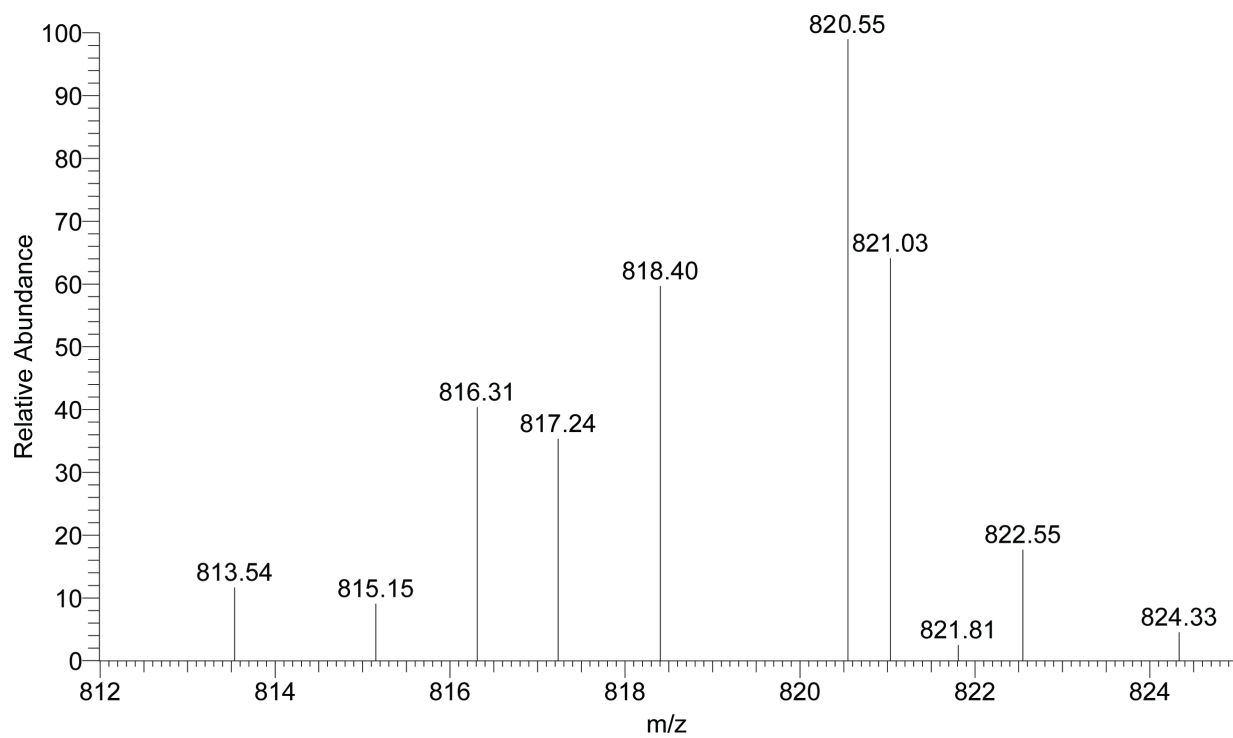
1H NMR (400 MHz, $CDCl_3$) δ 4.11 – 4.02 (m, 2H), 3.64 (t, $J = 6.6$ Hz, 2H), 3.47 – 3.27 (m, 2H), 2.92 – 2.41 (m, 7H), 2.39 – 2.18 (m, 4H), 1.76 – 1.13 (m, 72H), 0.90 – 0.86 (m, 9H).



Supplementary Fig. 75 | NMR Spectrum for N-SC-Lipid-3.



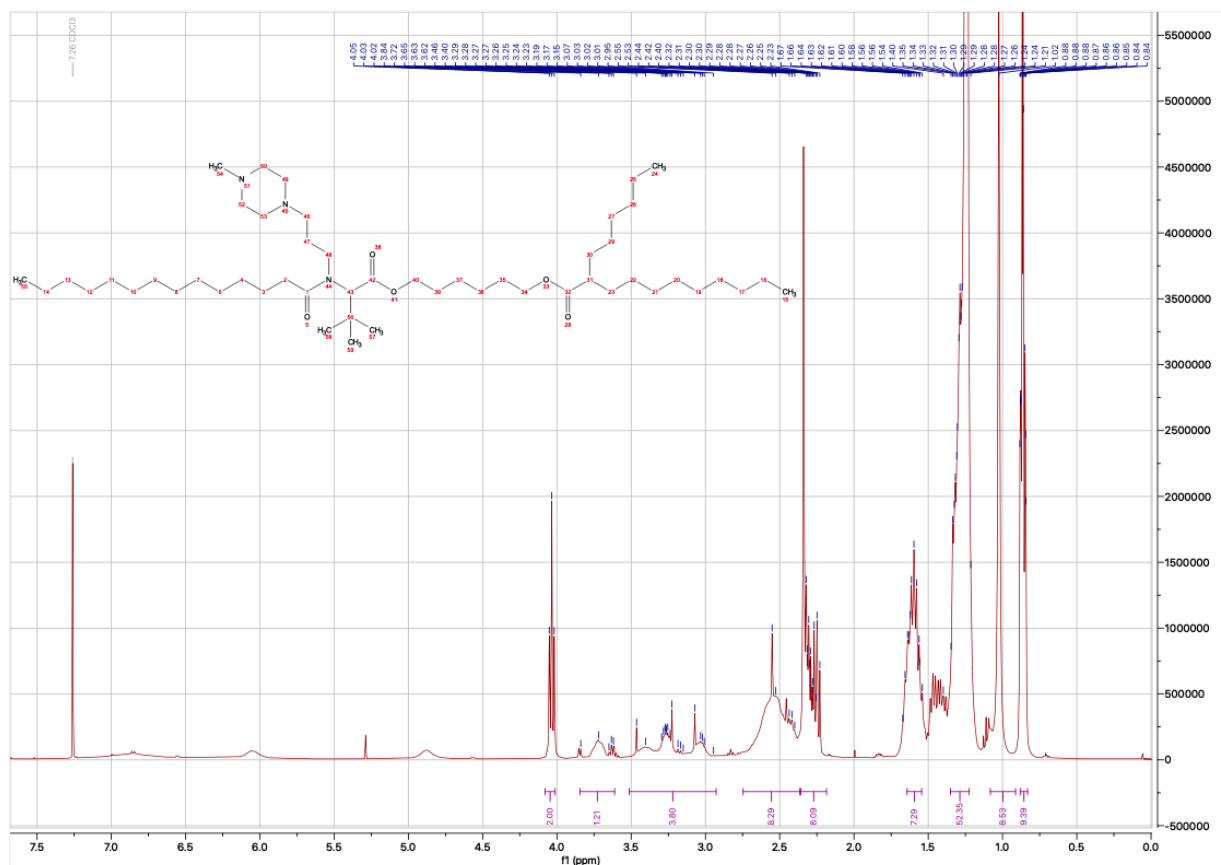
Supplementary Fig. 76 | NMR Spectrum for N-SC-Lipid-3 (δ 0.5-4.2 ppm region).



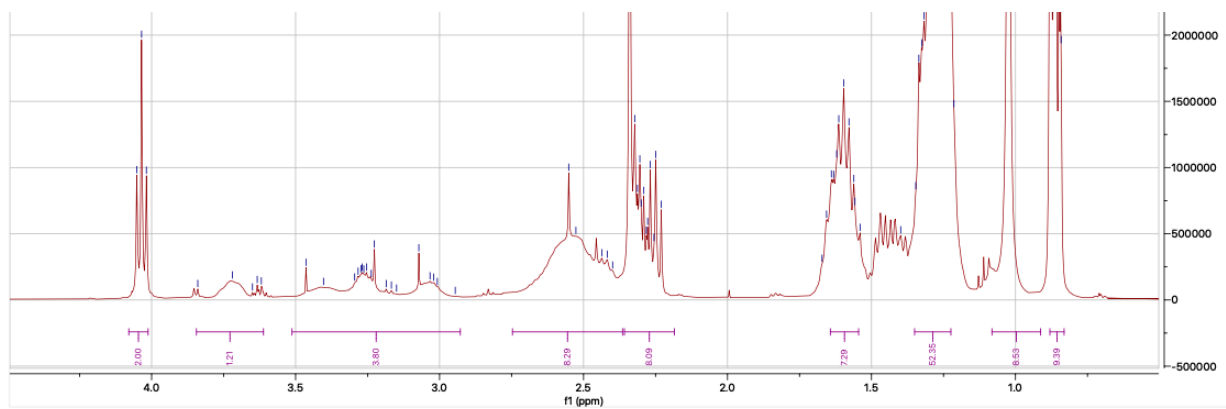
Supplementary Fig. 77 | Mass spectrometry spectrum of N-SC-Lipid-2.

N-LC-Lipid-1 (C₅₁H₉₉N₃O₅; MW: 834.37):

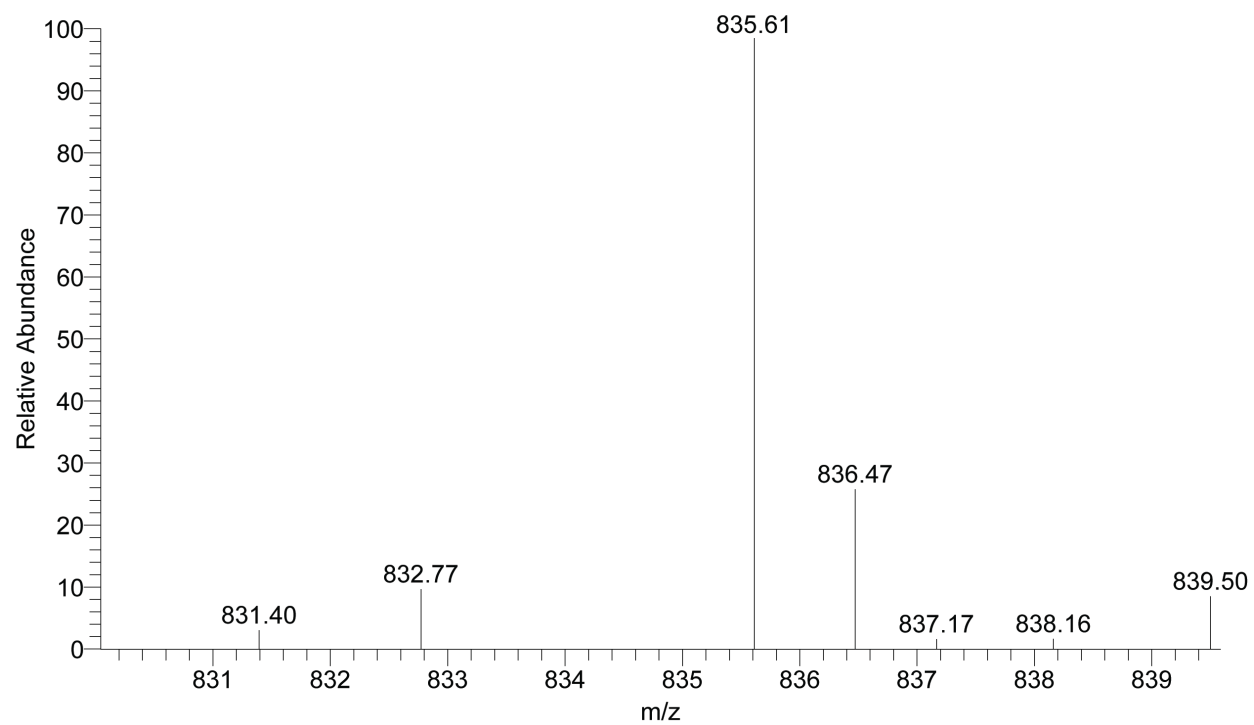
¹H NMR (400 MHz, CDCl₃) δ 4.03 (t, J = 6.7 Hz, 2H), 3.84 – 3.61 (m, 1H), 3.51 – 2.93 (m, 4H), 2.75 – 2.36 (m, 8H), 2.36 – 2.18 (m, 8H), 1.64 – 1.54 (m, 7H), 1.35 – 1.22 (m, 52H), 1.02 (s, 8H), 0.88 – 0.83 (m, 9H).



Supplementary Fig. 78 | NMR Spectrum for N-LC-Lipid-1.



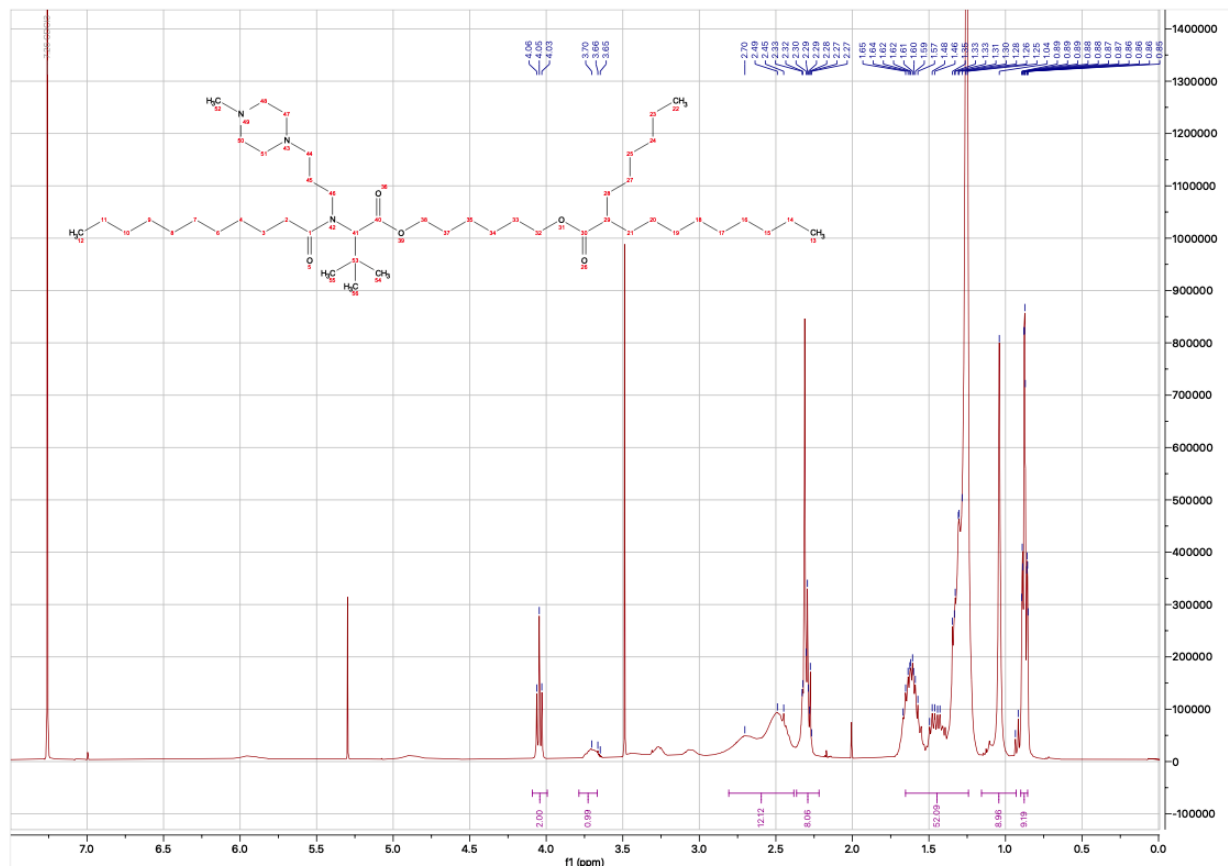
Supplementary Fig. 79 | NMR Spectrum for N-SC-Lipid-1 (δ 0.4-4.2 ppm region).



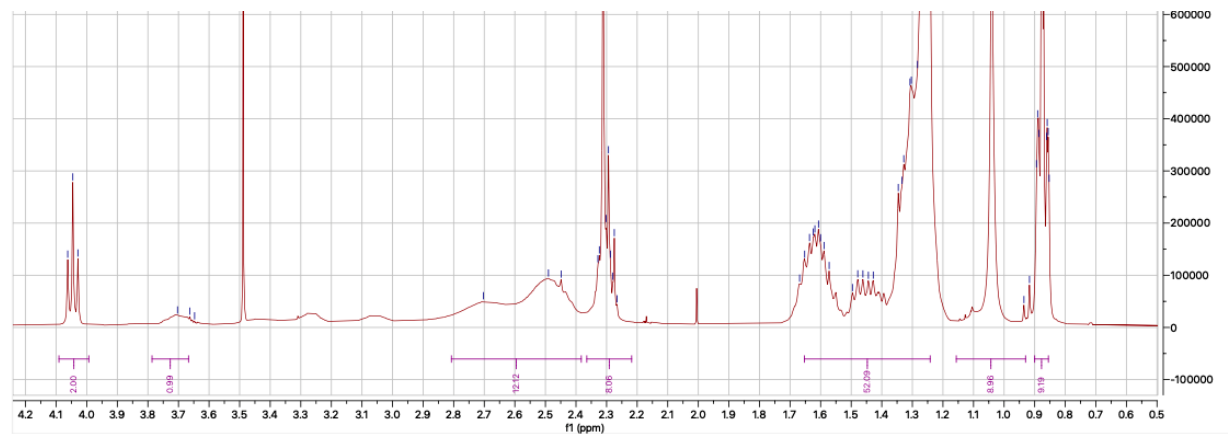
Supplementary Fig. 80 | Mass spectrometry spectrum of N-LC-Lipid-1.

N-LC-Lipid-2 ($C_{48}H_{93}N_3O_5$; MW: 792.29):

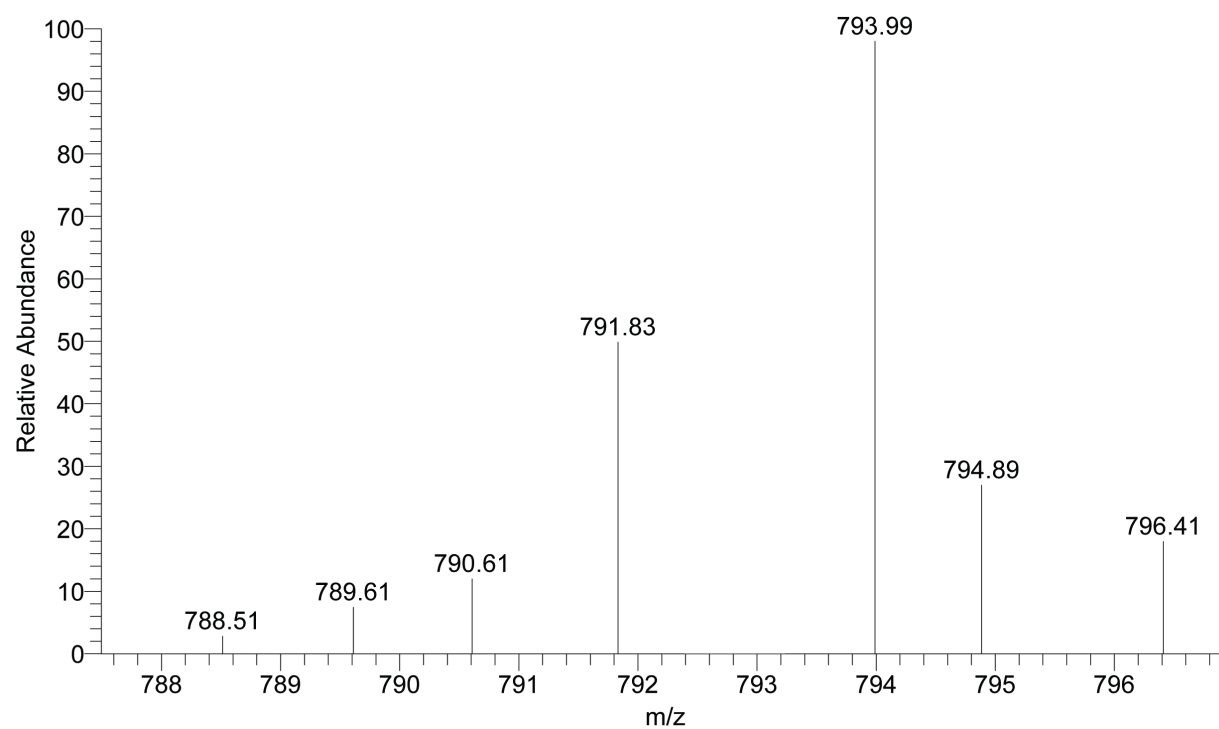
1H NMR (400 MHz, $CDCl_3$) δ 4.05 (t, J = 6.7 Hz, 2H), 3.79 – 3.67 (m, 1H), 2.81 – 2.38 (m, 12H), 2.36 – 2.22 (m, 8H), 1.65 – 1.24 (m, 52H), 1.04 (s, 9H), 0.90 – 0.86 (m, 9H).



Supplementary Fig. 81 | NMR Spectrum for N-LC-Lipid-2



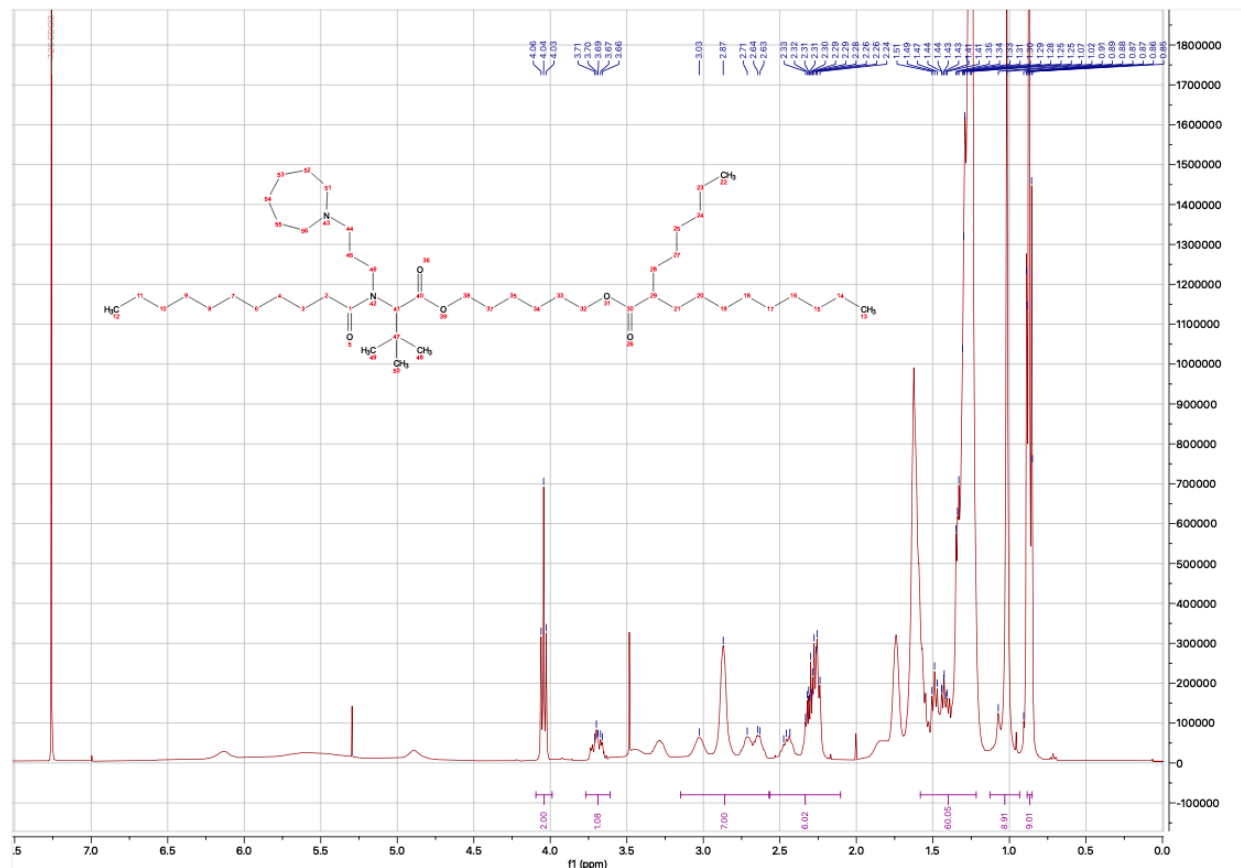
Supplementary Fig. 82 | NMR Spectrum for N-LC-Lipid-2 (δ 0.5-4.2 ppm region).



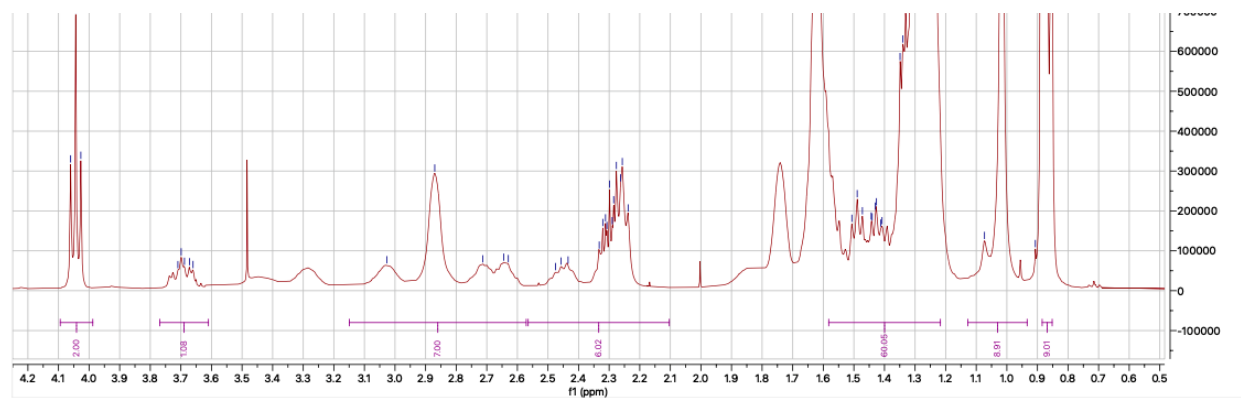
Supplementary Fig. 83 | Mass spectrometry spectrum of N-LC-Lipid-2.

N-LC-Lipid-3 ($C_{49}H_{94}N_2O_5$; MW: 791.30):

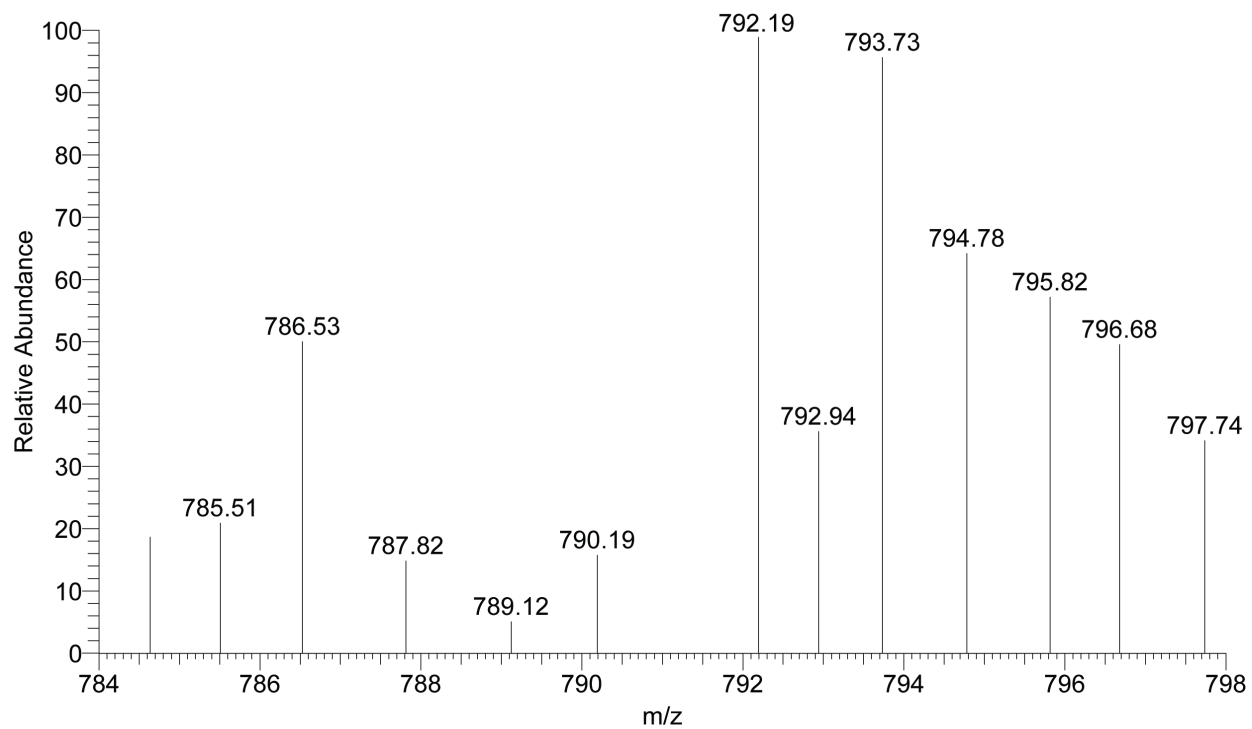
1H NMR (400 MHz, $CDCl_3$) δ 4.04 (t, $J = 6.7$ Hz, 2H), 3.77 – 3.61 (m, 1H), 3.15 – 2.57 (m, 7H), 2.56 – 2.10 (m, 6H), 1.58 – 1.22 (m, 60H), 1.02 (s, 9H), 0.88 – 0.85 (m, 9H).



Supplementary Fig. 84 | NMR Spectrum for N-LC-Lipid-3.



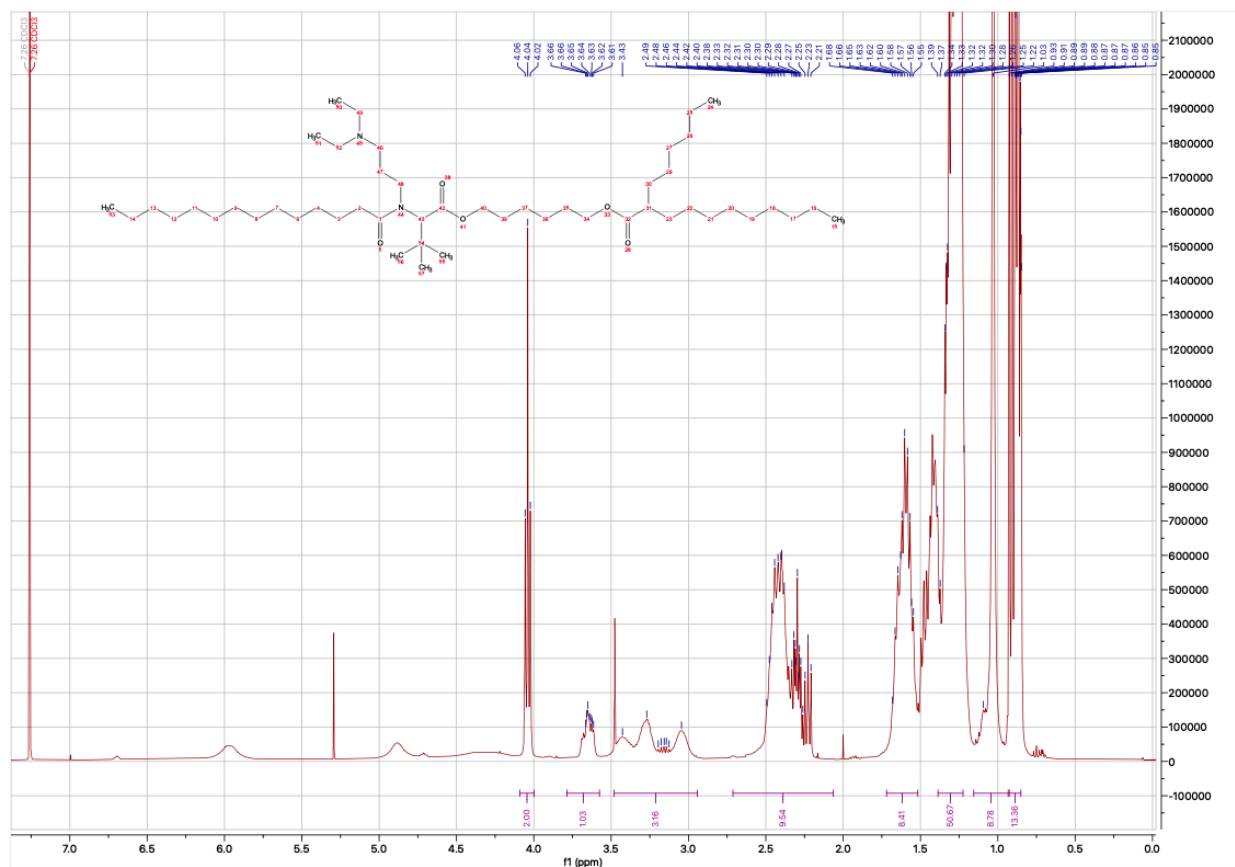
Supplementary Fig. 85 | NMR Spectrum for N-LC-Lipid-3 (δ 0.5-4.2 ppm region).



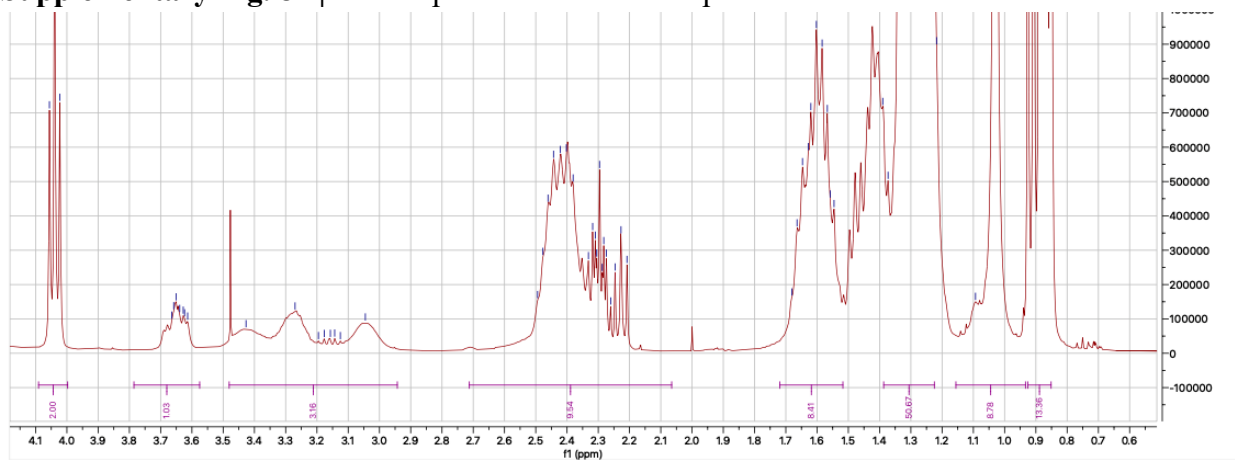
Supplementary Fig. 86 | Mass spectrometry spectrum of N-LC-Lipid-3.

N-LC-Lipid-1-Analogue-1 (C₅₀H₉₈N₂O₅; MW: 807.34):

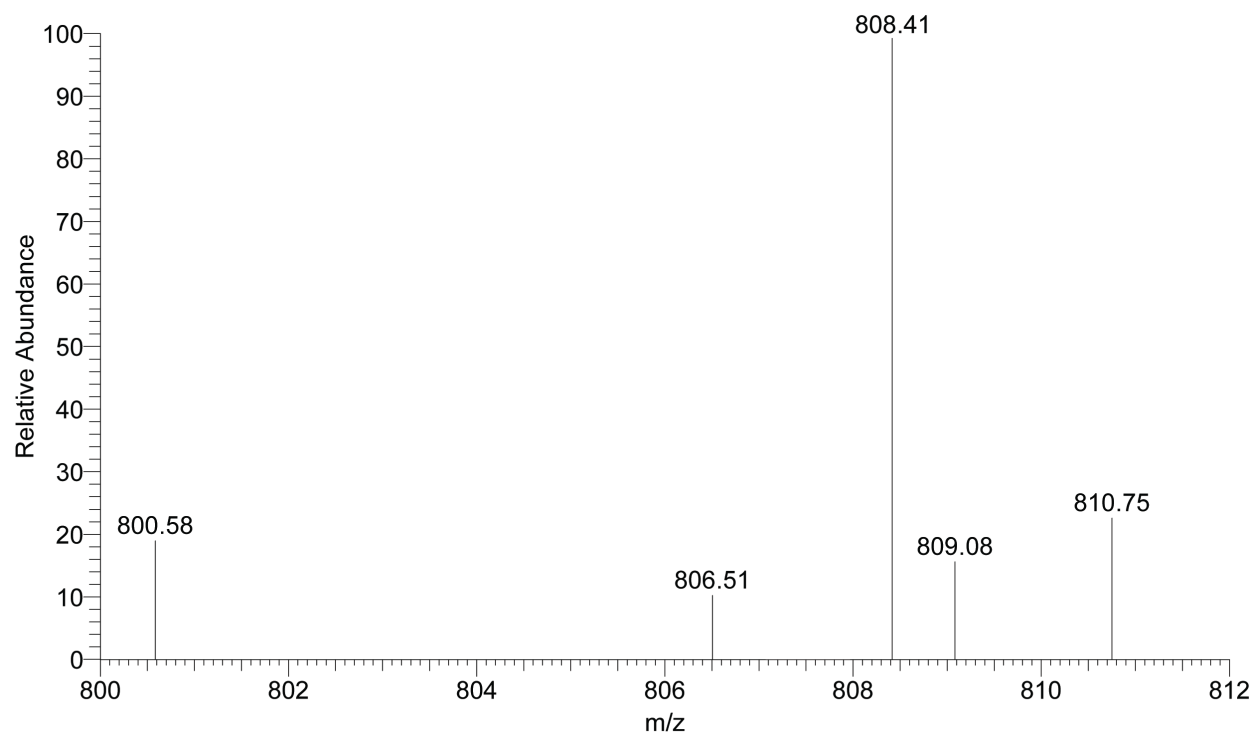
¹H NMR (400 MHz, CDCl₃) δ 4.04 (t, J = 6.7 Hz, 2H), 3.79 – 3.58 (m, 1H), 3.48 – 2.94 (m, 3H), 2.71 – 2.07 (m, 10H), 1.61 (ddt, J = 22.6, 15.2, 7.8 Hz, 8H), 1.27 (d, J = 20.2 Hz, 51H), 1.03 (s, 9H), 0.93 – 0.85 (m, 13H).



Supplementary Fig. 87 | NMR Spectrum for N-SC-Lipid-1.



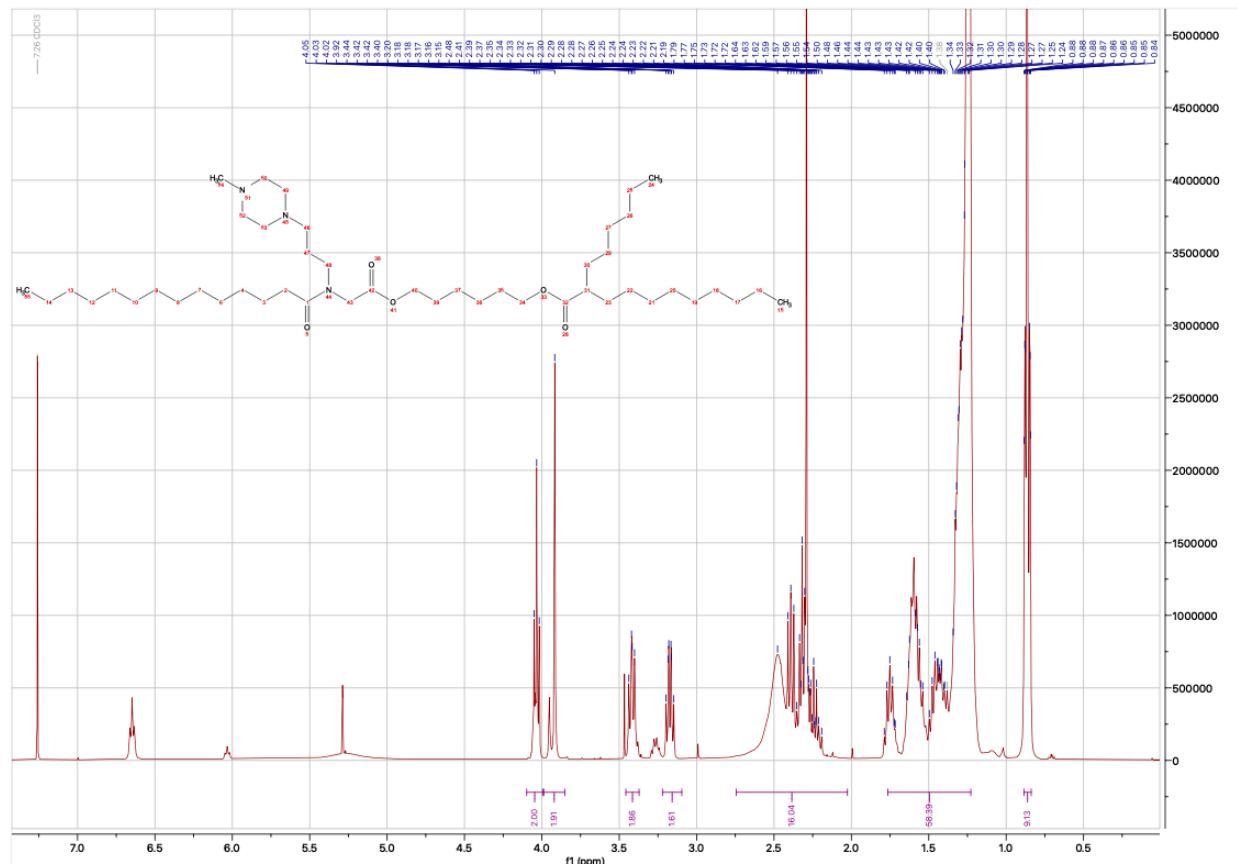
Supplementary Fig. 88 | NMR Spectrum for N-SC-Lipid-1 (δ 0.5-4.2 ppm region).



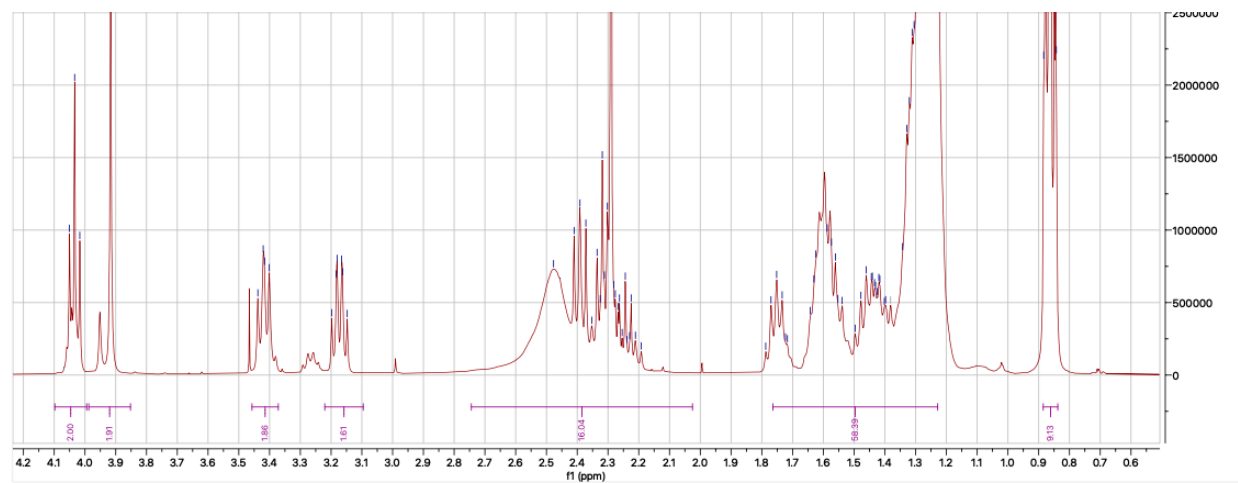
Supplementary Fig. 89 | Mass spectrometry spectrum of N-LC-Lipid-1-Analogue-1.

N-LC-Lipid-1-Analogue-2 (C₄₇H₉₁N₃O₅; MW: 778.26):

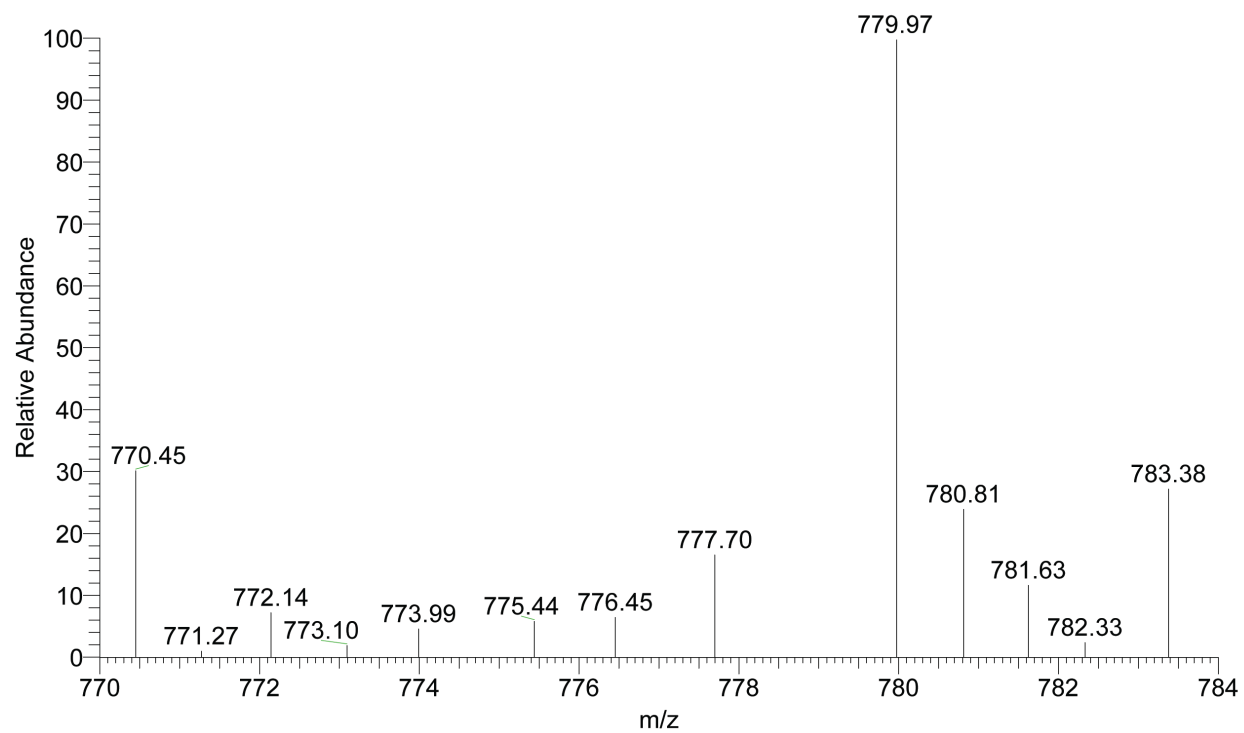
¹H NMR (400 MHz, CDCl₃) δ 4.03 (t, J = 6.7 Hz, 2H), 3.92 (s, 2H), 3.46 – 3.37 (m, 2H), 3.22 – 3.10 (m, 2H), 2.74 – 2.03 (m, 16H), 1.76 – 1.23 (m, 58H), 0.89 – 0.84 (m, 9H).



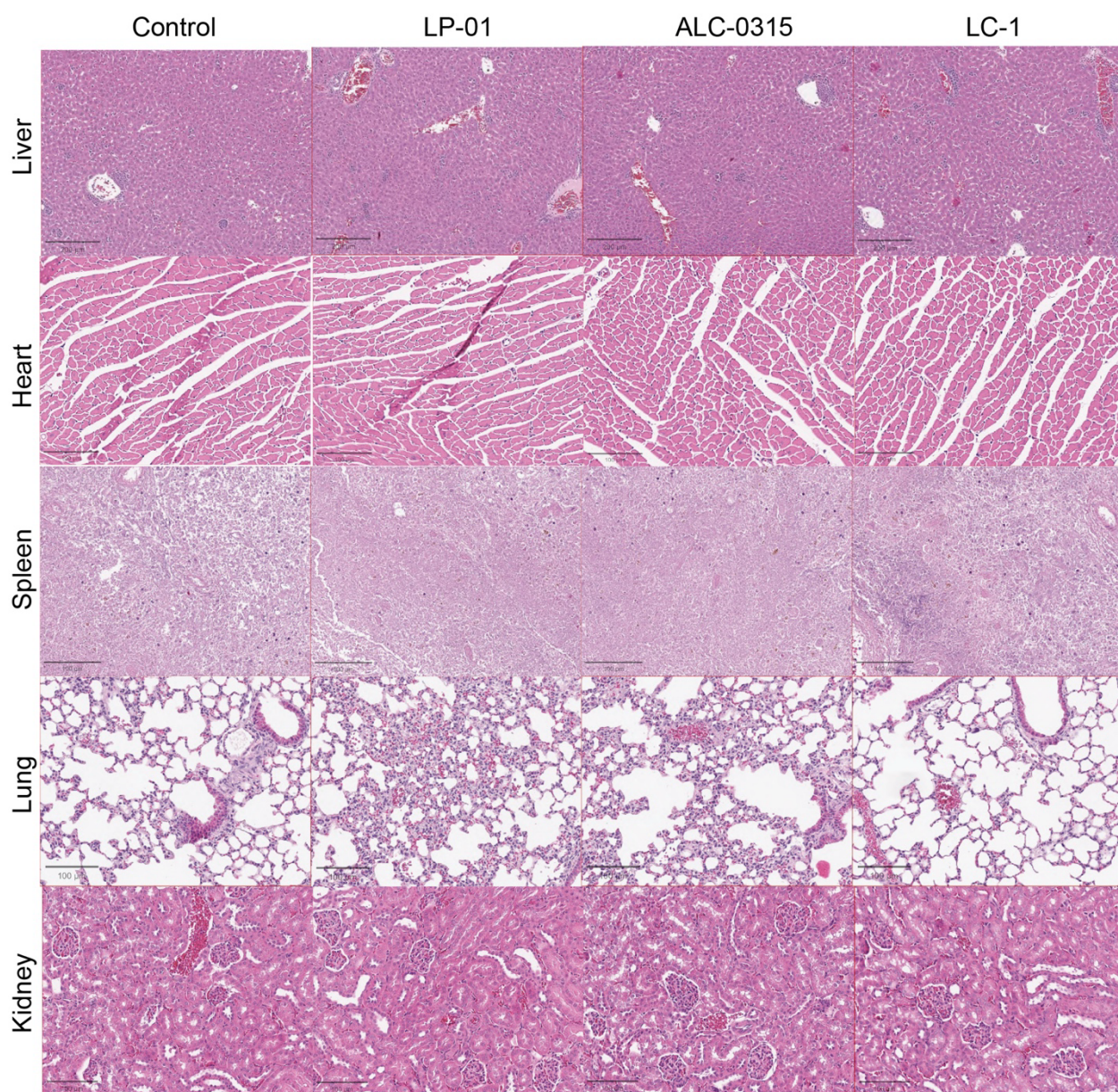
Supplementary Fig. 90 | NMR Spectrum for N-LC-Lipid-1-Analogue-2.



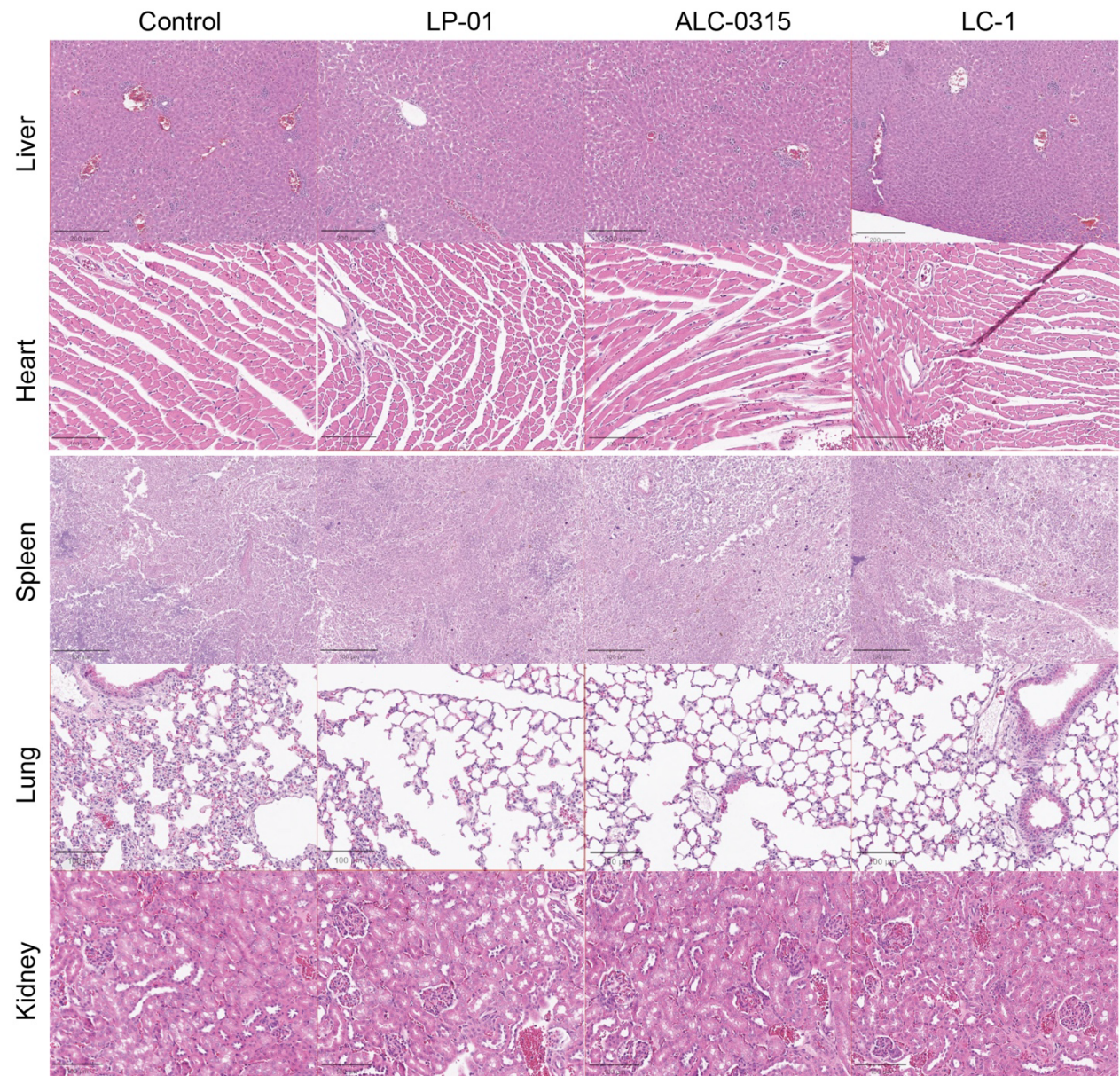
Supplementary Fig. 91 | NMR Spectrum for N-LC-Lipid-1-Analogue-2 (δ 0.5-4.2 ppm region).



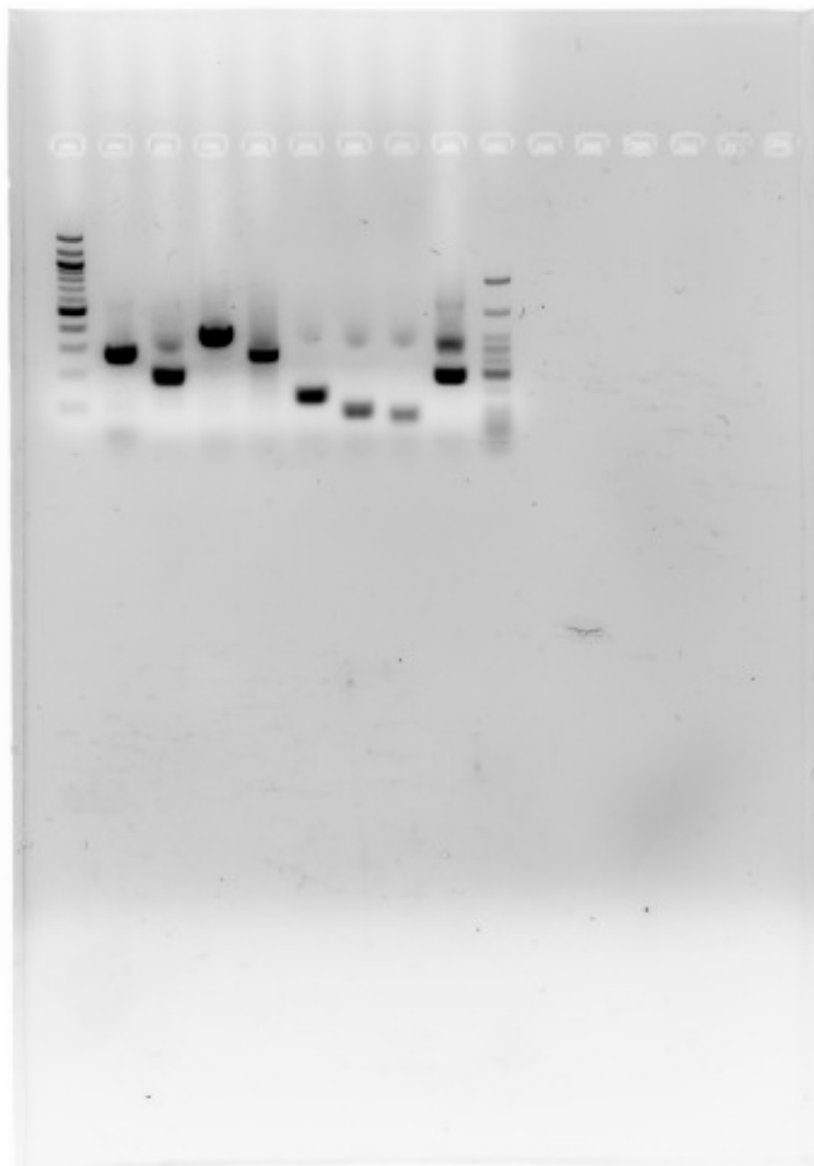
Supplementary Fig. 92 | Mass spectrometry spectrum of N-LC-Lipid-1-Analogue-2.



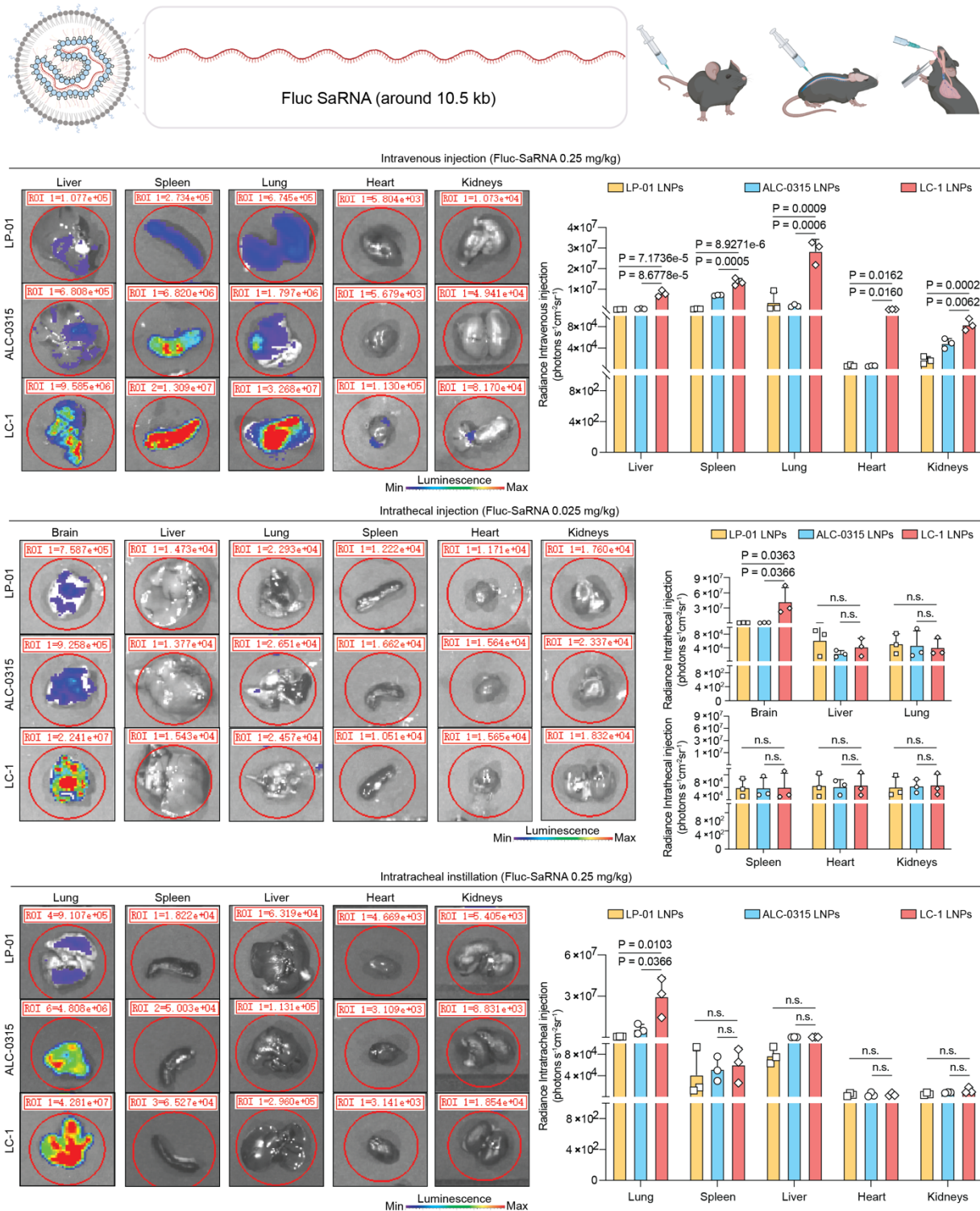
Supplementary Fig. 93 | Representative H&E staining of liver, lung, spleen, heart, and kidney from C57B/6 mice 60 day later after LNPs treatments. Scale bar is 100 μ m.



Supplementary Fig. 94 | Representative H&E staining of liver, lung, spleen, heart, and kidney from CFTR R553X mice 13 day later after LNPs treatments. Scale bar is 100 μ m.



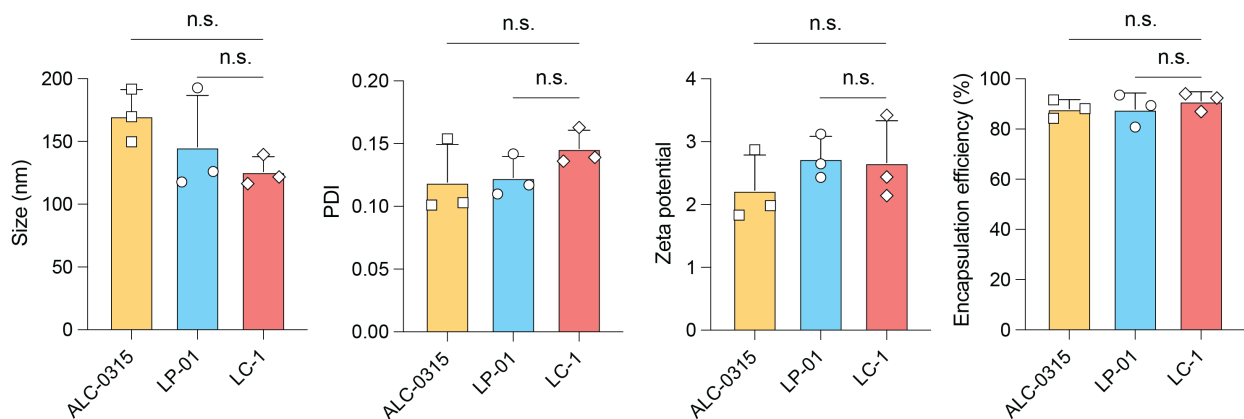
Supplementary Fig. 95 | Original gel image of Extended Fig. 1f.



Supplementary Fig. 96 | In vivo delivery of Fluc self-amplifying RNA (saRNA, ~10.5 kb) by LC-1 LNPs across multiple administration routes.

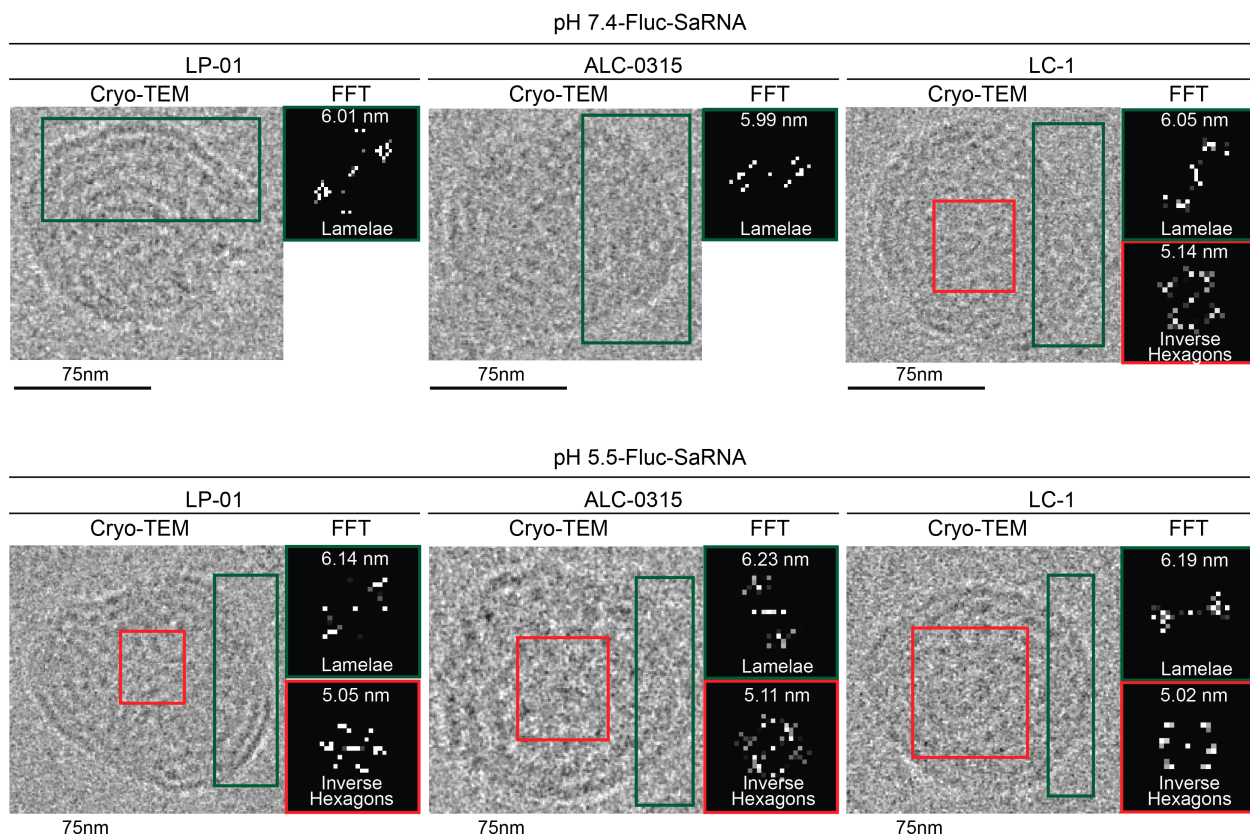
Schematic representation of Fluc saRNA (~10.5 kb) used to evaluate large-cargo delivery performance. Bioluminescence imaging and corresponding quantification following intravenous

(top, 0.25 mg kg⁻¹), intrathecal (middle, 0.125 mg kg⁻¹), and intratracheal (bottom, 0.25 mg kg⁻¹) administration of saRNA formulated in LP-01, ALC-0315, or LC-1 LNPs. Representative organ images (liver, spleen, lung, heart, kidney, and brain where applicable) and radiance quantification are shown. Data are presented as mean $\pm$ s.d. (n = 3 biological replicates). Statistical significance was determined by one-way ANOVA with multiple comparisons.



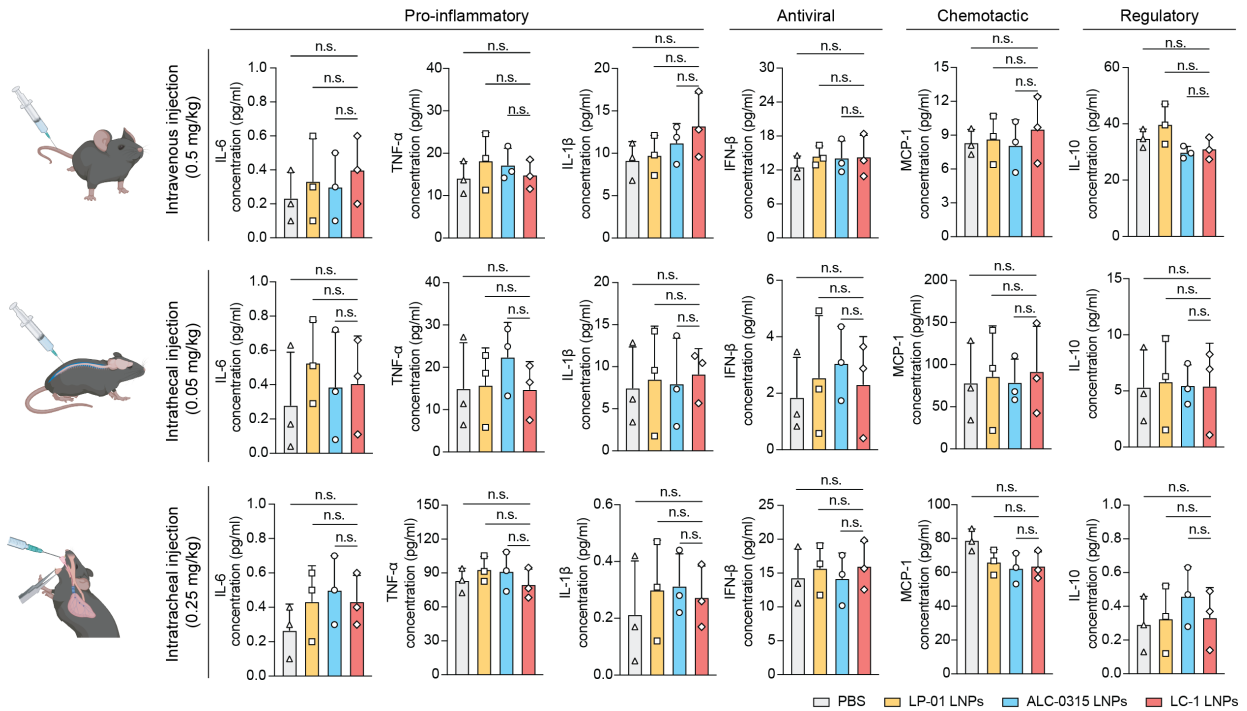
Supplementary Fig. 97 | Physicochemical characterization of saRNA-loaded LNP formulations.

Hydrodynamic diameter, polydispersity index (PDI), zeta potential, and encapsulation efficiency of Fluc self-amplifying RNA (saRNA) formulated in ALC-0315, LP-01, or LC-1 LNPs. No statistically significant differences were observed among groups (n.s.). Data are presented as mean $\pm$ s.d. (n = 3 independent experiments); statistical analysis was performed using one-way ANOVA.

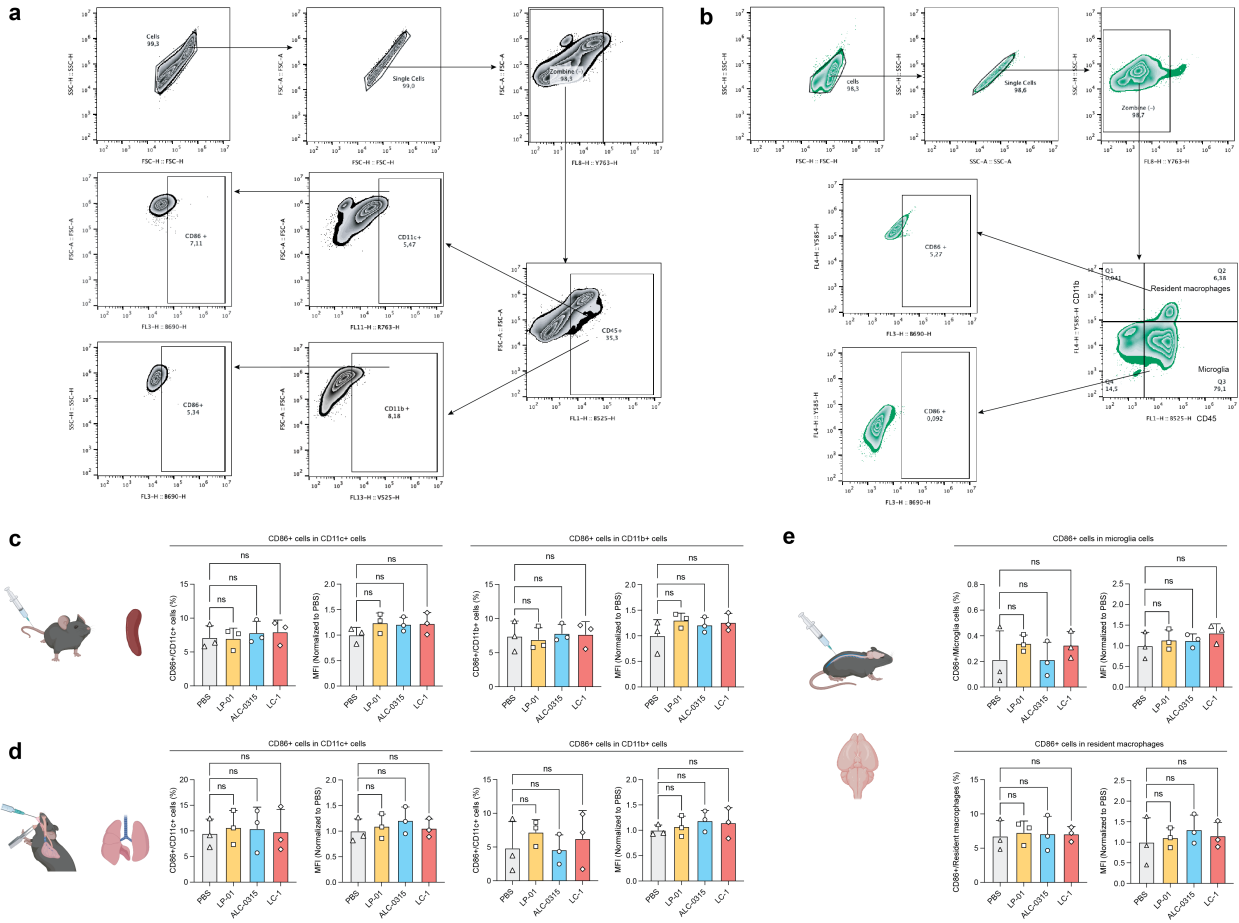


Supplementary Fig. 98 | Cryo-TEM analysis of saRNA-loaded LNPs at neutral and acidic pH.

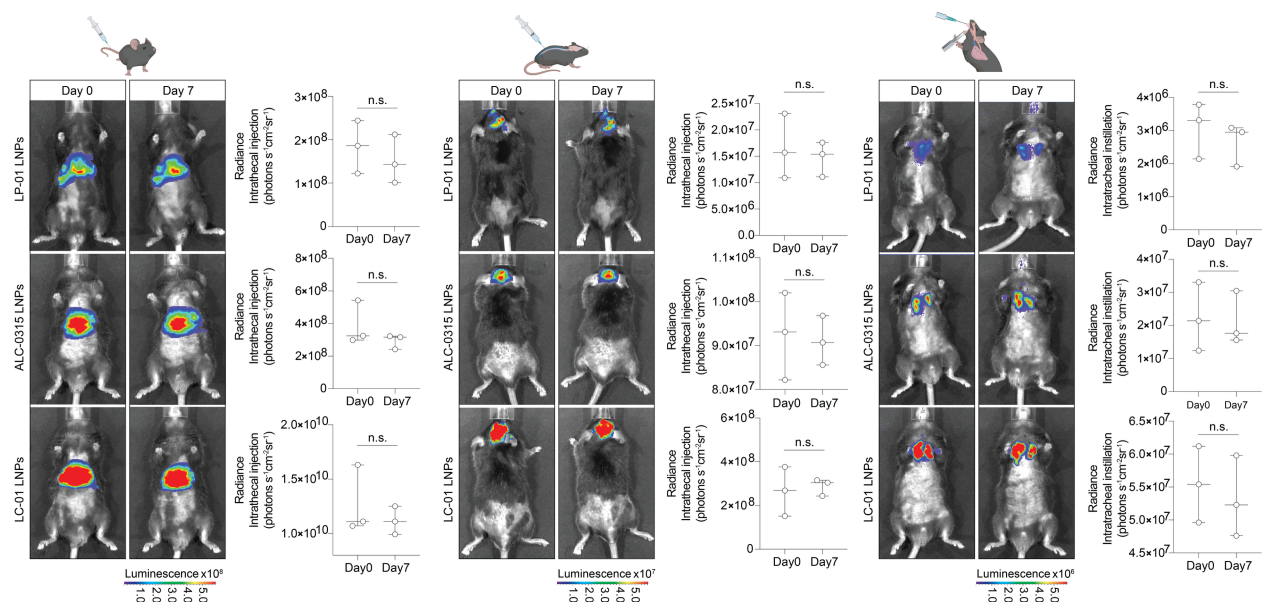
Representative cryo-TEM images and corresponding fast Fourier transform (FFT) analysis of Fluc self-amplifying RNA (saRNA, ~10.5 kb) formulated with LP-01, ALC-0315, or LC-1 at pH 7.4 (top) and pH 5.5 (bottom). Representative images from three independently prepared LNP batches are shown; similar results were obtained across all batches.



Supplementary Fig. 99 | Cytokine profiling following LC-1 LNP administration via different routes. Quantification of representative pro-inflammatory, antiviral, chemotactic, and regulatory cytokines following intravenous (top), intratracheal (middle), or intrathecal (bottom) administration of PBS, LP-01 LNPs, ALC-0315 LNPs, or LC-1 LNPs. Across all delivery routes, LC-1 LNPs did not induce statistically significant elevations in cytokine levels compared with PBS controls and showed responses comparable to benchmark formulations. Data are presented as mean $\pm$ s.d. ($n = 3$ biological replicates). Statistical analysis was performed using one-way ANOVA; n.s., not significant.



Supplementary Fig. 100 | Flow cytometry analysis of innate immune cell activation following LNP administration. (a) Representative gating strategy for splenic dendritic cells and macrophages following intravenous administration. (b) Representative gating strategy for brain immune populations following intrathecal administration. (c) Quantification of CD86⁺ cells and CD86 mean fluorescence intensity (MFI) in splenic CD11c⁺ dendritic cells and CD11b⁺ macrophages after intravenous injection of PBS, LP-01, ALC-0315, or LC-1 LNPs. (d) Quantification of CD86 expression in lung CD11c⁺ and CD11b⁺ immune cells following intratracheal administration. (e) Quantification of CD86⁺ cells and MFI in microglia and resident macrophages following intrathecal administration. Data are presented as mean $\pm$ s.d. (n = 3 biological replicates). Statistical analysis was performed using one-way ANOVA; n.s., not significant.



Supplementary Fig. 101 | Evaluation of adaptive immune responses following repeated LNP administration. Representative bioluminescence imaging and radiance quantification at Day 0 and Day 7 following repeated administration of mRNA formulated in LP-01, ALC-0315, or LC-1 LNPs. Radiance values (photons s⁻¹ cm⁻² sr⁻¹) were quantified for each animal at the indicated time points. Data are presented as mean ± s.d. (n = 3 biological replicates); statistical analysis was performed using paired comparisons as indicated. n.s., not significant.

Table-1. List of DNA sequences used as templates for mRNA constructs

mRNA Component	DNA Sequence
ABE8.8-m-NanoLuc mRNA	CleancapT7–5'UTR–ABE8.8-m–P2A–NanoLuc–SV40–3'UTR TAATACGACTCACTATAAGGGGACATCGTAGAGAGTCGTAAGAAAAATCTAT AGCAGAAAGTCAGCGGTAGACGACGGCATAGCATCCAACgccgccaccatgaaacggacagc cgacgggaagcgagttcgagtcaccaagaagaagcggaagtcgccgaagtcgagtttcccatgagtagtgatgagacagcattg actctcgcaagagggctcgagatgaacgcgaggtgcccggtggggcagtagctgctcaacaatcgctaatcgcggaaggttg aatagggcaatcggaactccagacccactgcacatgcggaaatcatggcccttcgacagggagggctgtgatgcagaattatcgac ttatcgatgcgacgctgtacgtcacgttgaaccttgcgtaatgtgcgcgggagctatgattcactcccgattggacgagttgtattcggt gttcgcaacgccaagacgggtgccgcaggttcaatgatggacgtgctgcatcatccaggcatgaaccaccgggtagaatcacagaa ggcatattggcgacgaatgtgcggcgctgtgtgtctgttttttcgcatgccaggcggtgtttaacgccagaaaaagcacaatcct ctactgactctgtgtgttctctgtgtgttctagcggcagcgagactcccgggacctcagagtcggccacaccgaaagtctgtgtgtt cttctgtgtgtgtgtgacaagaagtacagcatcgccctggccatcgccaccaactctgtgggtgggctgtacaccgacgagtacaa ggtgcccgcaagaattcaaggtgtgtgggaacaccgaccggcacagcatcaagaagaacctgatcgagccctgctgttcgaca gcggcgaaacagccgagggccaccggctgaagagaaccggcagaagaatacaccagacggaagaaccggatctgtatctgc aagagatcttcagcaacgagatggcaaggtggacgacgcttctccacagactggaagagtccttctgtgtgaagaggataagaa gcacgagcggcaccctctctcgcaacatcgtggacgaggtggcctaccacgagaagtacccaccatctaccactgagaagaa aactggtggacagcaccgacaagggcgacctgcggctgatctatctggccctggccacatgatcaagttccggggccacttctgat cgagggcgacctgaaccccgacaacagcgacgtggacaagctgttcacagctggtgcagacctacaaccagctgttcgaggaaa accccatcaacgcagcggcggtggacgccaaggccatcgtctgcagactgagcaagagcagacggctggaaaatctgatcgcc cagctgcccggcgagaagaagaatggcctgttcggaaacctgattgccctgagcctggcgctgaccccaactcaagagcaactc gacctggccgaggtgccaactgcagctgagcaaggacacctacgacgacgacctggacaacctgtggccagatcgcgacc agtacggcgacctgttctgtggcgccaagaacctgtccgacgccatctgtgagcgacatctgagagtgaacaccgagatcacaa ggccccctgagcgctctatgatcaagagatacagcagcaccaccagacctgacctgctgaaagctctctgtcgcgacgagct gcctgagaagtacaagagattttctcgaccagagcaagaacggctacgcccgtacattgacggcgagccagccaggaagagtt ctacaagttcatcaagccatctggaagaatggacggcaccgaggaactgctgtgaagctgaacagagaggacctgtcgcgaa gcagcggaccttcgacaacggcagcatccccaccagatccacctgggagagctgcacgccattctgcggcgcgaggaagatttta cccattctgaaggacaaccgggaaaagatcgagaagatctgaccttcgcacatccctactacgtgggcccctctgtgccaggggaaa cagcagattcgcttgatgaccagaaagagcgaggaaccatcacccctggaacttcgaggaagtgtgtgacaaggcgcttccg cccagagcttcatcgagcggatgaccaacttcgataagaacctgcccaacgagaaggtgtgtcccaagcacagcctgtgtacgagt acttcacctgtataacgagctgaccaaaagtgaatactgtaccgaggggaatgagaagcccgccttctgagcggcgagcagaaaa aggccatcgtggacctgtgttcaagaccaaccggaaagtaccgtgaagcagctgaaagaggactactcaagaaaatcgagtgtt cgactcgtggaatctccggcgtggaagatcggttaacgcctccctgggcacataccacgatctgtgtaaaattatcaaggacaag gacttctgtgacaatgaggaaaacgaggacattctggaagatatctgtgtgacctgacactgtttgaggacagagagatgatcagg aacggctgaaaacctatgccacctgttcgacgacaagtgtgaagcagctgaagcggcgagatacaccggctggggcgaggctg agccgggaagctgatcaacggcatccgggacaagcagtcggcgcaagacaatcctggatttctgaagtccgacggcttcgccaacaga aacttcatgcagctgatccacgacgacagcctgaccttaagaggacatccagaaagcccagggtgtccggccaggggcgatagcctg cacgagcacattgccaatctggccggcagccccgccattaagaagggcatcctgcagacagtgaaggtgtgtggacgagctctgaa agtgtgtggcgccgacaaagcccgaacatctgtatcgaatggccagagagaaccagaccaccagaaggagcagaagaacag ccgcgagagaatgaagcggatcgaagagggcatcaaagagctgggcagccagatcctgaaagaacaccccggtgaaacaccca gttcgagaacgagaagctgtacctgtactacctgcagaatggcggggatgtacgtggaccaggaactggacatcaaccggctgtc cgactacgatgtggaccatctgtgctcagagcttctgaaggcagctccatcgacaacaaggtgtgaccagaagcgacaagaac cggggcaagagcgacaacgtgccctccgaagaggtcgtgaagaagatgaagaactactggcgcgagctgtgaacccaagctga ttaccagagaaagttgacaatctgaccaaggccgagagaggcgccctgagcgaactggataaggcgcttcatcaagagacag ctggtggaaacccggcagatcacaaagcacgtggcacagatcctggactccggatgaacactaagtacacgagagatgacaagct gatccgggaagtgaagtgtatcaccctgaagtcgaagctgtgttcggaaggtatttcaggttttacaaggtgcgcgagatcaa caactaccacagccacgacgcctactgaacgcgtgtgtgggaaccgcctgatcaaaaagtacctaaagctggaaagcgagtt ctgtacggcgactacaaggtgtacgacgtgcgggaagatgatcgccaagagcgagcaggaatcgcgcaaggctaccgccaagtact tcttctacagcaacatcatgaacttttcaagaccgagattacctggccaacggcgagatccggaagcgcgctctgtacgagacaac ggcgaaacccggggagatcgtgtgggataaggccggggtatttccaccgtgcggaaagtgtgagcatccccaaagtgaatatctg aaaaagaccgaggtgcagacagcggttcagcaaaaggtctatcctgccaaaggaacagcgataagctgatccgcaagaagaa ggactgggaccctaagaagtacggcggttcgacagccccaccgtggcctattctgtctgtgtgtggccaaagtggaaaagggcaa gtccaagaaactgaagagtgtaagagctgtgtgggatccatcatggaaagaagcagcttcgagaagaatccatcgacttctg

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Table-2. The sequence of sgRNA used for Ai9, LumA mice, PCSK9, and CFTR R553X.

Name	Sequence (5'-3')	PAM
sgTOM	AAGTAAAACCTCTACAAATG	TGG
sgLumA	GCCTCATTGATTAACGCCCA	GGG
mPCSK9	CCCATACCTTGGAGCAACGG	CGG
hPCSK9	CCCGCACCTTGGCGCAGCGG	TGG
hCFTR	TTGCTCATTGACCTCCACTC	NG

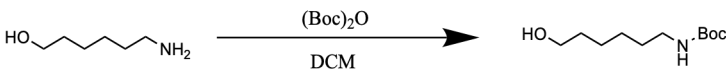
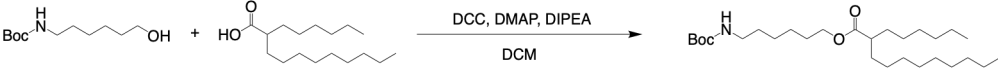
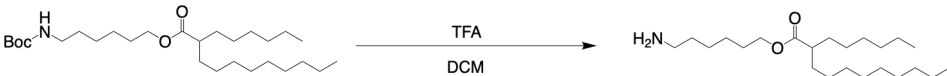
Table-3. The sequence of primers used for Sanger sequencing.

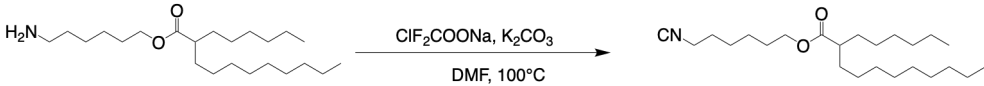
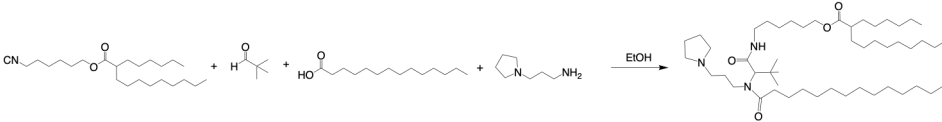
Name	Sequence (5'-3')
mPCSK9 forward	ATGAAGAGCTGATGCTCGCC
mPCSK9 reverse	CAGGGCTGCACTTAGAGACCACC
hPCSK9 forward	GCTAGCCTTGCGTTCCGA
hPCSK9 reverse	CACTTCCTCTCTTACATGGGGG
hR553X forward	AGAAGGAAGATGTGCCTTTCA
hR553X reverse	CAAATGCTTGCTAGACCAATAATTAGT

Table-4. Quantification of SAXS results.

mRNA type	pH	LNPs	Lamellae phase (%)	Inverse Hexagons phase (%)
Fluc mRNA	7.4	LP-01	95.2%	4.8%
		ALC-0315	81.5%	16.5%
		LC-1	64.2%	35.8%
	5.5	LP-01	57.4%	42.6%
		ALC-0315	44.8%	55.2%
		LC-1	24.2%	75.8%
ABE mRNA	7.4	LP-01	94.6%	5.4%
		ALC-0315	92.8%	7.2%
		LC-1	72.5%	27.5%
	5.5	LP-01	75.7%	24.3%
		ALC-0315	59.2%	40.8%
		LC-1	40.4%	59.6%
ABE mRNA	7.4	LC-1	72.5%	27.5%
		LC-1-Analogue-1	75.7%	24.3%
		LC-1-Analogue-2	89.2%	10.8%
	5.5	LC-1	40.4%	59.6%
		LC-1-Analogue-1	45.2%	54.8%
		LC-1-Analogue-2	64.3%	35.7%

Table-5. Detailed synthetic steps of LC-1.

Step		Details					
Step 1	1.1 Reaction Scheme						
	1.2 Reaction Ratio	Compound	CAS	Molecular Weight	Moles (mol)	Weight (g)	Equivalents (eq.)
		Amino-6-hexanol	4048-33-3	117.19	0.2133	25	1.0
		Di-tert-butyl-dicarbonate	24424-99-5	218.25	0.2176	47.49	1.02
	1.3 Reaction Operation	6-Amino-1-hexanol (25 g, 0.2133 mol) was dissolved in 300 mL anhydrous DCM and cooled in an ice bath. A solution of di-tert-butyl dicarbonate (47.49 g, 0.2176 mol) in 100 mL DCM was added dropwise under stirring. The reaction mixture was allowed to warm to room temperature and stirred for 2 h.					
	1.4 Post-reaction processing	Reaction completion was monitored by TLC. The solvent was removed under reduced pressure to afford the Boc-protected intermediate, which was used directly in the next step.					
Step 2	2.1 Reaction Scheme						
	2.2 Reaction Ratio	Compound	CAS	Molecular Weight	Moles (mol)	Weight (g)	Equivalents (eq.)
		6-boc-Amino-hexanol		217.31	0.1	21.731	1.0
		2-Hexylundecanoic acid	72056-18-9	270.45	0.1	27.045	1.0
		DCC	25952-53-8	191.7	0.15	28.7553	1.5
		DMAP	1122-58-3	122.17	0.01	1.2217	0.1
		DIPEA	7087-68-5	129.25	0.2	25.85	2
		DCM				400ml	
	2.3 Reaction Operation	2-Hexylundecanoic acid (25.64 g, 0.10 mol) was dissolved in 300 mL anhydrous DCM under ice bath conditions. DCC (28.76 g), DMAP (1.22 g), and DIPEA (25.85 g) were added sequentially. After stirring for 10 min, a solution of 6-Boc-amino-hexanol (21.73 g) in 100 mL DCM was added dropwise. The mixture was stirred overnight at room temperature.					
	2.4 Post-reaction processing	The reaction mixture was diluted with DCM, washed twice with water and once with saturated brine, dried over anhydrous Na ₂ SO ₄ , concentrated under reduced pressure, and purified by silica gel chromatography (PE/EA, 0-20%).					
Step 3	3.1 Reaction Scheme						
		Compound	CAS	Molecular Weight	Moles (mol)	Weight (g)	Equivalents (eq.)

	3.2 Reaction Ratio	6-((tert-butoxycarbonyl)amino)hexyl 2-hexylundecanoate		469.75	0.07	30	1.0
		Trifluoroacetic acid	76-05-1			200 ml	
		DCM				200 ml	
	3.3 Reaction Operation	The protected intermediate (30 g) was dissolved in 200 mL DCM. Trifluoroacetic acid (200 mL) was added slowly at room temperature. The mixture was stirred for 2 h.					
	3.4 Post-reaction processing	After TLC confirmation, solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate, washed with saturated NaHCO ₃ and brine, dried over Na ₂ SO ₄ , and concentrated..					
Step 4	4.1 Reaction Scheme						
	4.2 Reaction Ratio	Compound	CAS	Molecular Weight	Moles (mol)	Weight (g)	Equivalents (eq.)
		6-aminoheptyl 2-hexylundecanoate		369.61	0.05	18.48	1.0
		Sodium Chlorodifluoroacetate	1895-39-2	152.46	0.1	15.24	2
		K ₂ CO ₃	584-08-7	138.21	0.1	13.82	2
		DMF				250 ml	
	Reaction Operation	The amine intermediate was dissolved in DMF (250 mL). K ₂ CO ₃ and sodium chlorodifluoroacetate were added. The reaction flask was degassed (vacuum/N ₂ cycles, 3×) and heated at 100 °C for 18 h..					
Step 5	Post-reaction processing	After TLC confirmation, the mixture was filtered through Celite, diluted with ethyl acetate, washed three times with brine, dried over Na ₂ SO ₄ , concentrated, and purified by silica gel chromatography (PE/EA, 0–20%).					
	5.1 Reaction Scheme						
	5.2 Reaction Ratio	Compound	CAS	Molecular Weight	Moles (mol)	Weight (g)	Equivalents (eq.)
		6-isocyanohexyl 2-hexylundecanoate		379.63	5	1.90	1.0
		Pivaldehyde	630-19-3	86.13	5	0.43	1.0
		3-(Pyrrolidin-1-yl)propan-1-amine	23159-07-1	128.22	5	0.52	1.2
		Myristic acid	544-63-8	228.37	5	1.1419	1
		Anhydrous EtOH				50 ml	
	5.3 Reaction Operation	Pivalaldehyde was dissolved in anhydrous methanol. The amine was added and stirred for 10 min. Myristic acid was added and stirred for an additional 10 min. The isocyanide intermediate was then added. The mixture was stirred at room temperature overnight.					

	5.4 Post-reaction processing	The reaction mixture was concentrated under reduced pressure and purified by silica gel chromatography (DCM/MeOH, 0-15%).
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